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(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER [US/US]; 2500 North State Street, Jackson, MS 39216–4505 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: PILETZ, John, E. [US/US]; 14 Peachtree Lane, Madison, MS 39110 (US). IVANOV, Tina, R. [CA/US]; 2590 N. State Street, Jackson, MS 39216 (US).

(74) Agent: CHEEK, Warren, M., Jr.; Wenderoth, Lind & Ponack, Suite 800, 2033 "K" Street, N.W., Washington, DC 20006 (US).

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(54) Title: DNA MOLECULES ENCODING IMIDAZOLINE RECEPTIVE POLYPEPTIDES AND POLYPEPTIDES ENCODED THEREBY

(57) **Abstract**

A genomic DNA encoding a human imidazoline receptor is described. cDNAs encoding the receptor and fragments thereof are also provided. An amino acid sequence predicted to be 120,000 MW for nearly the entire protein is identified, as well as a middle fragment believed to contain the imidazoline binding site of the receptor. The protein is highly unique in its sequence and may represent the first in a novel family of receptor proteins. Methods of cloning the cDNA and expressing the imidazoline receptor in a host cell are described. Methods of preparing antibodies against the transfected protein are also described. Also, a screening method for identifying additional subtypes of this receptor are identified. Also, screening methods for identifying drugs that interact with the imidazoline receptor are described.

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DNA MOLECULES ENCODING IMIDAZOLINE RECEPTIVE POLYPEPTIDES AND POLYPEPTIDES ENCODED THEREBY

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is directed to DNA molecules encoding imidazoline receptive polypeptides, preferably encoding human imidazoline receptive polypeptides, that can be used as an imidazoline receptor (abbreviated IR). In addition, transcript(s) and protein sequences are predicted from the DNA clones. The invention is also directed to a genomic DNA clone designated as JEP-1A. The cDNA clones according to the invention comprise cDNA homologous to portion(s) of this genomic clone; including 5A-1 cDNA, cloned by the inventors that established the open-reading frame for translation of mRNA from the gene, and established the immunoreactive properties of its polypeptide sequence in an expression systems. Also, the invention relates to cDNA clone EST04033, which is another clone identified to contain cDNA sequences from the JEP-1A gene, and of which the 5A-1 is a part, that encodes an active fragment of the IR polypeptide in transfection assays, and the protein sequences thereof. The invention also relates to methods for producing such genomic and cDNA clones, methods for expressing the IR protein and fragments, and uses thereof.

2. Description of Related Art

It is believed that brainstem imidazoline receptors possess binding site(s) for therapeutically relevant imidazoline compounds, such as clonidine and idazoxan. These drugs represent the first generation of ligands discovered for the binding site(s) of imidazoline receptors. However, clonidine and idazoxan were developed based on their high affinity for α_2-adrenergic receptors. Second generation ligands, such as moxonidine, possess somewhat improved selectivity for IR over α_2-adrenergic receptors, but more selective compounds for IR are needed.

An imidazoline receptor clone is of particular interest because of its potential utility in identifying novel pharmaceutical agents having greater potency and/or more selectivity than currently available ligands have for imidazoline receptors. Recent technological advances permit pharmaceutical companies to use combinatorial chemistry techniques to rapidly screen a cloned receptor for ligands (drugs) binding thereto. Thus, a cloned imidazoline receptor would be of significant value to a drug discovery program.

Until now, the molecular nature of imidazoline receptors remains unknown. For instance, no amino acid sequence data for a novel IR, e.g., by N-terminal sequencing, has been reported. Three different techniques have been described in the literature by three different laboratories to visualize imidazoline-selective binding proteins (imidazoline receptor candidates) using gel electrophoresis. Some important consistencies have emerged from these results despite the diversity of the techniques employed. On the other hand,

multiple protein bands have been identified, which suggests heterogeneity amongst imidazoline receptors. These reports are discussed below.

Some of the abbreviations used hereinbelow, have the following meanings:

α_2AR	Alpha-2 adrenoceptor
BAC	Bovine adrenal chromaffin
ECL	Enhanced chemiluminescence (protein detection procedure)
EST	Expressed Sequence Tag (a one-pass cDNA documentation without identification)
I-site	Any imidazoline-receptive binding site (e.g., encoded on IR)
IR_1	Imidazoline receptor subtype$_1$
IR-Ab	Imidazoline receptor antibody
I_2Site	Imidazoline binding subtype$_2$
kDa	Kilodaltons (molecular size)
MAO	monoamine oxidase
MW	molecular weight
NRL	European abbreviation for RVLM (see below)
PC-12	Phaeochromocytoma-12 cells
^{125}PIC	[^{125}I]p-iodoclonidine
PKC	Protein Kinase C
RVLM	Rostral Ventrolateral Medulla in brainstem
SDS	sodium dodecyl sulfate gel electrophoresis

Reis et al. [Wang et al., Mol. Pharm., 42: 792-801 (1992); Wang et al., Mol. Pharm., 43: 509-515 (1993)] were the first to characterize an imidazoline-selective binding protein and to demonstrate it as having MW = 70 kDa. This was accomplished using bovine cells (BAC), which lack an α_2AR [Powis & Baker, Mol. Pharm., 29:134-141 (1986)]. The 70 kDa imidazoline-selective protein in those studies had high affinities for both idazoxan and p-aminoclonidine affinity chromatography columns and was eluted by another imidazoline compound (phentolamine). Unfortunately, those investigators failed to isolate sufficient 70 kDa protein to determine its other biochemical properties. To date, no one has reported the complete purification of an imidazoline receptor protein.

Likewise, no amino acid sequences have been reported for IR.

Their 70 kDa protein was used by Reis and co-workers to raise "I-site binding antiserum", designated herein as Reis antiserum. The term "I-site" refers to the imidazoline binding site, presumably defined within the imidazoline receptor protein. Reis antiserum was prepared by injecting the purified protein into rabbits [Wang et al, 1992]. The first immunization was done subcutaneously with the protein antigen (10 μg) emulsified in an equal volume of complete Freund's adjuvant, and the next three booster shots were given at 15-day intervals with incomplete Freund's adjuvant. The polyclonal antiserum has been mostly characterized by immunoblotting, but radioimmunoassays (RIA) and/or conjugated assay procedures, i.e., ELISA assays, are also conceivable [see "Radioimmunoassay of Gut Regulatory Peptides: Methods in Laboratory Medicine," Vol. 2, chapters 1 and 2, Praeger Scientific Press, 1982].

The present inventors and others [Escriba et al., Neurosci. Lett. 178: 81-84 (1994)] have characterized the Reis antiserum in several respects. For instance, the present inventors have discovered that human platelet immunoreactivity with Reis antiserum is mainly confined to a single protein band of MW ≈ 33 kDa, although a trace band at ≈ 85 kDa was also observed. The ≈ 33 and ≈ 85 kDa bands were enriched in plasma membrane fractions as expected for an imidazoline receptor. Furthermore, the intensity of the ≈ 33 kDa band was found to be positively correlated with non-adrenergic ^{125}PIC Bmax values at platelet IR_1 sites in samples from the same

subjects, with an almost one-to-one slope factor. In addition, the nonadrenergic ^{125}PIC binding sites on platelets were discovered by the present inventors to have the same rank order of affinities as IR_1 binding sites in brainstem [Piletz and Sletten, J.Pharm. & Exper. Therap., 267: 1493-1502 (1993)]. The platelet ≈ 33 kDa band may also be a product of a larger protein, since in human megakaryoblastoma cells, which are capable of forming platelets in tissue cultures, an ≈ 85 kDa immunoreactive band was found to predominate.

Immunoreactivity with Reis antiserum does not appear to be directed against human α_2AR and/or MAO A/B. This is a significant point because α_2AR and MAO A/B have previously been cloned and also bind to imidazolines. The present inventors have obtained selective antibodies and recombinant preparations for α_2AR and MAO A/B, and these proteins do not correspond to the ≈ 33, 70, or 85 kDa putative IR_1 bands. Thus, there is substantial evidence that, at least in human platelets, the Reis antiserum is IR_1 selective.

Another antiserum was raised by Drs. Dontenwill and Bousquet in France [Greney et al., Europ. J. Pharmacol., 265: R1-R2 (1994); Greney et al., Neurochem. Int., 25: 183-191 (1994); Bennai et al., Annals NY Acad. Sci., 763:140-148 (1995)] against polyclonal antibodies for idazoxan (designated Dontenwill antiserum). This anti-idiotypic antiserum inhibits ^{3}H-clonidine but not ^{3}H-rauwolscine (α_2-selective) binding sites in the brainstem, suggesting it also interacts with IR_1 [Bennai et al., 1995]. As shown in Fig. 1, human RVLM (same as NRL) membrane fractions displayed bands of ≈ 41 and 44 kDa, as

detected by the present inventors using this anti-idiotypic antiserum.

The present inventors have found that the bands of MW ≈ 41 and 44 kDa detected by Dontenwill antiserum may be derived from an ≈ 85 kDa precursor protein, similar to that occurring in platelet precursor cells. An 85 kDa immunoreactive protein is obtained in fresh rat brain membranes only when a cocktail of 11 protease inhibitors is used. Also, as shown in Fig. 1, it is found that Reis antiserum detects the ≈ 41 and 44 kDa bands in human brain when fewer protease inhibitors are used. Additionally, the Dontenwill antiserum weakly detects a platelet ≈ 33 kDa band. Thus, the present inventors have hypothesized that the ≈ 41 and 44 kDa immunoreactive proteins may be alternative breakdown products of an ≈ 85 kDa protein, as opposed to the platelet ≈ 33 kDa breakdown product.

In summary, the main conclusion from the above results is that, despite vastly different origins, the Reis and Dontenwill antisera both detect identical bands in human platelets, RVLM, and hippocampus.

Using yet another technique, a photoaffinity imidazoline ligand, ^{125}AZIPI, has also been developed to preferentially label I_2-imidazoline binding sites [Lanier et al., J.Biol.Chem., 268: 16047-16051 (1993)]. The ^{125}AZIPI photoaffinity ligand was used to visualize ≈ 55 kDa and ≈ 61 kDa binding proteins from rat liver and brain. It is believed that the ≈ 61 kDa protein is probably MAO, in agreement with other findings [Tesson et al., J.Biol.Chem., 270: 9856-9861 (1995)] showing that MAO proteins bind certain imidazoline

compounds. The different molecular weights between these bands and those detected immunologically by the present inventors is one of many pieces of evidence that distinguishes IR_1 from I_2 sites.

To the inventors' knowledge and as described herein, we are first to clone the gene, cDNAs and fragments thereof encoding a protein with the immunological and ligand binding properties expected of an IR. On this basis, we are first to identify the nucleotide sequences of DNA molecules encoding an imidazoline receptor and active fragments thereof, and the first to determine the amino acid sequence of an imidazoline receptor and active fragments thereof. The polypeptides described herein are clearly distinct from α_2AR or MAO A/B proteins.

SUMMARY OF THE INVENTION

The present invention involves various cDNA clones (ie., 5A-1 and EST04033) and a genomic clone (JEP-1A) which are directed to an isolated polypeptide(s) that is receptive to (bind to) imidazoline compound(s), and can be used to identify other compounds of interest. Currently available imidazoline compounds in this context are p-iodoclonidine and moxonidine. Initially, the inventors detected a polypeptide expressed by their cDNA clone (5A-1 isolated from a human hippocampus cDNA library) that immunoreacted with Reis antiserum and/or Dontenwill antiserum. The DNA sequence of the 5A-1 clone is encapsulated within a portion of the other clones (EST04033 and JEP-1A genomic clone).

In one aspect of the invention, a polypeptide includes a 651 amino acid sequence as shown in SEQ ID No. 5. This polypeptide is predicted from non-plasmid cDNA in EST04033; a clone which the inventors showed possesses sequences inclusive of 5A-1. Furthermore, transfection of EST04033 into COS cells yielded imidazoline receptivity by radioligand binding assays (described in detail later). Other imidazoline receptive proteins homologous to this polypeptide are also contemplated. Such polypeptide(s) generally have a molecular weight of about 50 to 80 kDa. More particularly, one can have a molecular weight of about 70 kDa.

In another aspect of this invention, a polypeptide includes a 390 amino acid sequence as shown in SEQ ID No. 6. This represents the polypeptide predicted from the non-plasmid DNA of the original 5A-1 clone. Such a polypeptide generally has a molecular weight of about 35 to 50 kDa. More particularly, it can have a molecular weight of about 43 kDa.

DNA molecules encoding aforementioned imidazoline-receptive polypeptide(s) are also contemplated. Such a DNA molecule, e.g., a cDNA derived from mRNA, can contain a nucleotide sequence encoding the 651 amino acid sequence shown in SEQ ID No. 5. Thus, a DNA molecule containing the 1954 base pairs (b.p.) (1954 b.p. encodes 651 amino acids) nucleotide sequence shown in SEQ ID No. 2 is contemplated. This represents the coding sequence for the polypeptide predicted by EST04033 transfections. In another embodiment, a DNA molecule includes the longer nucleotide sequence shown in SEQ ID No. 3. This represents the cDNA predicted to have been

translated + not predicted to have been translated in transfections experiments of EST04033.

In another embodiment of the invention, a DNA molecule contains a nucleic acid sequence encoding the amino acid sequence shown in SEQ ID No. 6. In another aspect, it can include the 1171 b.p. nucleic acid sequence shown in SEQ ID No. 4. The 1171 b.p. nucleic acid sequence shown in SEQ ID No. 4 is the 5A-1 non-plasmid DNA.

The nucleic acid sequence of the genomic clone encoding the imidazoline receptor is further shown in SEQ ID No. 21. The nucleic acid and amino acid sequence of the predicted transcript (ie., cDNA) can be predicted from the description hereinbelow. The polypeptide encoded by the genomic DNA is shown in SEQ ID No. 22.

Sequence similarity with the sequences indicated in SEQ ID protocols of the attached Sequence Listing is defined in connection with the present invention as a very close structural relationship of the relevant sequences with the sequences indicated in the respective SEQ ID protocols. To determine the sequence similarity, in each case the structurally mutually corresponding sections of the sequence of the SEQ ID protocol and of the sequence to be compared therewith are superimposed in such a way that the structural correspondence between the sequences is a maximum, account being taken of differences caused by deletion or insertion of individual sequence members (DNA-codon or amino acid respectively), and being compensated by appropriate shifts in

sections of the sequences. The sequence similarity in % results from the number of sequence members which now correspond to one another in the sequences ("homologous positions") relative to the total number of members contained in the sequences of the SEQ ID protocols. Differences in the sequences may be caused by variation, insertion or deletion of sequence members. Additionally in DNA sequences, different DNA-codons encoding for the same amino acid are considered identical in the context of the present invention. For amino acid sequences, conservative amino acid substitutions encoded by their corresponding DNA-codons, as well as naturally occurring homologs of the sequences, are considered within the context of sequence similarity.

DNA molecules of substantial homology (≥ 75 %) are an implicit aspect of this sort of invention. As will be discussed later, the inventors have already identified two possible splice variants in the amino acid coding sequence. In addition, artificially mutated receptor cDNA molecules can be routinely constructed by methods such as site-directed polymerase chain reaction-mediated mutagenesis [Nelson and Long, Anal. Biochem. 180: 147-151 (1989)]. It is commonly appreciated that highly homologous mutants frequently mimic their natural receptor. A study by Kjelsberg et al. [J. Biol. Chem. 267: 1430-1433 (1992)] showed that all 20 amino acid substitutions produce an active receptor at a single site in the α_{1b}-adrenergic receptor. RNA molecules of ≥ 75 % complementarity to an instant DNA molecule, e.g., an mRNA molecule (sense) or a complementary cRNA molecule (antisense),

are a further aspect of the invention.

A further aspect of the invention is for a recombinant vector, as well as a host cell transfected with the recombinant vector, wherein the recombinant vector contains at least one of the nucleotide sequences shown in SEQ ID Nos. 1-4, or sequences predicted by the genomic clone, or nucleotide sequences ≥ 75 % homologous thereto.

A method of producing an imidazoline receptor protein is another aspect of the invention. Such a method entails transfecting a host cell with an aforementioned vector, and culturing the transfected host cell in a culture medium to generate the imidazoline receptor.

A method for producing homologous imidazoline receptor proteins, and the proteins produced thereby, are also considered an aspect of this invention.

A significant further aspect of the invention is a method of screening for a ligand that binds to an imidazoline receptor. Such a method can comprise culturing an above-mentioned transfected cell in a culture medium to express imidazoline receptor proteins, followed by contacting the proteins with a labelled ligand for the imidazoline receptor under conditions effective to bind the labelled ligand thereto. The imidazoline receptor proteins can then be contacted with a candidate ligand, and any displacement of the labelled ligand from the proteins can be detected. Displacement of labelled ligand signifies that the candidate ligand is a ligand for the imidazoline receptor. These steps could be performed on intact host cells, or on proteins

isolated from the cell membranes of the host cells.

The invention will now be described in more detail with reference to specific examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a comparison of Reis antiserum (lane 1, 1:2000 dilution) and Dontenwill antiserum (lane 2, 1:5000 dilution) immunoreactivities for human NRL (same as RVLM) and hippocampus, as discussed in Example 1.

Fig. 2 depicts a comparison of Reis antiserum (1:15,000 dilution) and Dontenwill antiserum (1:20,000 dilution) immunoreactivities for plaques isolated from the human hippocampal cDNA library used in cloning as discussed in Example 2. The plaques contain the initial clone, designated herein as 5A-1, in a third stage of purification.

Fig. 3 depicts the restriction map of the EST04033 cDNA clone.

Fig. 4 depicts a competitive binding assay between ^{125}I-labelled p-iodoclonidine (PIC) and various ligands for the imidazoline receptor on membranes expressed in COS cells transfected with the EST04033 cDNA clone, as discussed in Example 4.

Fig. 5 depicts the prediction of introns and exons of the genomic clone (as analyzed by the GENESCAN program and verified by the available CDNAS).

Fig. 6 depicts the distribution of MRNA homologous to our CDNA in human adult tissues (bar graph) and the two species of MRNA (6 and 9.5 kb).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is concerned with multiple aspects of an imidazoline receptor protein, and DNA molecules encoding the same, and fragments thereof, which have now been discovered.

First, a polypeptide having imidazoline binding activity has been identified, which contains the putative active site for binding, as discussed hereinafter. Although polypeptide(s) described herein has a binding affinity for an imidazoline compound, it may also have an enzymatic activity, such as do catalytic antibodies and ribozymes. In fact, one such domain within our protein predicts a cytochrome p450 activity (described later).

Exemplary "binding" polypeptides are those containing either of the amino acid sequences shown in SEQ ID Nos. 5 or 6 (with the amino acid sequence predicted by EST04033 given in SEQ ID No. 5). Functionally equivalent polypeptides are also contemplated, such as those having a high degree of homology with such aforementioned polypeptides, particularly when they contain the Glu-Asp-rich region described hereinafter which we believe may define an active imidazoline binding site.

A polypeptide of the invention can be formed by direct chemical synthesis on a solid support using the carbodiimide method [R. Merrifield, JACS, 85: 2143 (1963)]. Alternatively, and preferably, an instant polypeptide can be produced by a recombinant DNA technique as described herein and elsewhere [e.g., U.S. Patent No. 4,740,470 (issued to Cohen and Boyer), the disclosure of which is incorporated herein by reference],

followed by culturing transformants in a nutrient broth.

Second, a DNA molecule of the present invention encodes aforementioned polypeptide. Thus, any of the degenerate set of codons encoding an instant polypeptide is contemplated. A particularly preferred coding sequence is the 1954 b.p. sequence set forth in SEQ ID No. 2, which has now been discovered to be a nucleotide sequence that encodes a polypeptide capable of binding imidazoline compound(s). In another embodiment, a DNA molecule includes the 3318 b.p. nucleotide sequence shown in SEQ ID No. 3. This latter sequence is the entire EST04033 insert. It includes the nucleotide sequence of SEQ ID No. 2 which was predicted to have been translated into protein in the transfection experiments.

In another embodiment of the invention, a DNA molecule contains a nucleic acid sequence encoding the amino acid sequence (390 residues) shown in SEQ ID No. 6. This amino acid sequence corresponds to that derived from direct sequencing of the 5A-1 clone and represents a fragment of the native protein. The 5A-1 DNA molecule is defined by the 1171 b.p. nucleic acid sequence shown in SEQ ID No. 4.

A DNA molecule of the present invention can be synthesized according to the phosphotriester method [Matteucci et al., JACS, 103: 3185 (1988)]. This method is particularly suitable when it is desired to effect site-directed mutagenesis of an instant DNA sequence, whereby a desired nucleotide substitution can be readily made. Another method for making an instant DNA molecule is by simply growing cells

transformed with plasmids containing the DNA sequence, lysing the cells, and isolating the plasmid DNA molecules. Preferably, an isolated DNA molecule of the invention is made by employing the polymerase chain reaction (PCR) [e.g., U.S. Patent No. 4,683,202 (issued to Mullis)] using synthetic primers that anneal to the desired DNA sequence, whereby DNA molecules containing the desired nucleotide sequence are amplified. Also, a combination of the above methods can be employed, such as one in which synthetic DNA is ligated to CDNA to produce a quasi-synthetic gene [e.g., U.S. Patent No. 4,601,980 (issued to Goeddel et al.)].

A further aspect of the invention is for a vector, e.g., a plasmid, that contains at least one of the nucleotide sequences shown in SEQ ID Nos. 1-4 or those predicted by the genomic clone in SEQ ID No. 21. Whenever the reading frame of the vector is appropriately selected, the vector encodes an IR polypeptide of the invention. Hence, as well as full-length protein, fragments of the native IR protein are contemplated; as well as fusion proteins that incorporate an amino acid sequence as described herein. Also, a vector containing a nucleotide sequence having a high degree of homology with any of SEQ ID Nos. 1-4 or 21 is contemplated within the invention, particularly when it encodes a protein having imidazoline binding activity.

A recombinant vector of the invention can be formed by ligating an afore-mentioned DNA molecule to a preselected expression plasmid, e.g., with T4 DNA ligase. Preferably, the plasmid and DNA molecule are provided with cohesive

(overlapping) terminii, with the plasmid and DNA molecule operatively linked (i.e., in the correct reading frame).

Another aspect of the invention is a host cell transfected with a vector of the invention. Relatedly, a protein expressed by a host cell transfected with such a vector is contemplated, which protein may be bound to the cell membrane. Such a protein can be identical with an aforementioned polypeptide, or it can be a fragment thereof, such as when the polypeptide has been partially digested by a protease in the cell. Also, the expressed protein can differ from an aforementioned polypeptide, as whenever it has been subjected to one or more post-translational modifications. For the protein to be useful within the context of the present invention, it should exhibit imidazoline binding capacity.

A method of producing an imidazoline receptor protein is another aspect of the invention, which entails transfecting a host cell with an aforementioned vector, and culturing the transfected host cell in a culture medium to generate the imidazoline receptor. The receptor molecule can undergo any post-translational modification(s), including proteolytic decomposition, whereby its structure is altered from the basic amino acid residue sequence encoded by the vector. A suitable transfection method is electroporation, and the like.

With respect to transfecting a host cell with a vector of the invention, it is contemplated that a vector encoding an instant polypeptide can be transfected directly in animals. For instance, embryonic stem cells can be transfected, and the cells can be manipulated in embryos to produce transgenic

animals. Methods for performing such an operation have been previously described [Bond et al., Nature, 374:272-276 (1995)]. These methods for expressing an instant CDNA molecule in either tissue culture cells or in animals can be especially useful for drug discovery.

Possibly the most significant aspect of the present invention is in its potential for affording a method of screening for a ligand (drug) that binds to an imidazoline receptor. Such a method comprises culturing an above-mentioned host cell in a culture medium to express an instant imidazoline receptive polypeptide, then contacting the polypeptides with a labelled ligand, e.g., radiolabelled p-iodoclonidine, for the imidazoline receptor under conditions effective to bind the labelled ligand thereto. The polypeptides are further contacted with a candidate ligand, and any displacement of the labelled ligand from the polypeptides is detected. Displacement signifies that the candidate ligand actually binds to the imidazoline receptor. These steps could be performed on intact host cells, or on proteins isolated from the cell membranes of the host cells.

Typically, a suitable drug screening protocol involves preparing cells (or possibly tissues from transgenic animals) that express an instant imidazoline receptive polypeptide. In this process, categories of chemical structure are systematically screened for binding affinity or activation of the receptor molecule encoded by the transfected CDNA. This process is currently referred to as combinatorial chemistry. With respect to the imidazoline receptor, a number of

commercially available radioligands, e.g., ^{125}PIC, can be used for competitive drug binding affinity screening.

An alternative approach is to screen for drugs that elicit or block a second messenger effect known to be coupled to activation of the imidazoline receptor, e.g., moxonidine-stimulated arachidonic acid release. Even with a weak binding affinity or activation by one category of chemicals, systematic variations of that chemical structure can be studied and a preferred compound (drug) can be deduced as being a good pharmaceutical candidate. Identification of this compound would lead to animal testing and upwards to human trials. However, the initial rationale for drug discovery becomes vastly improved with an instant cloned imidazoline receptor.

Along these lines, a drug screening method is contemplated in which a host cell of the invention is cultured in a culture medium to express an instant imidazoline receptive polypeptide. Intact cells are then exposed to an identified agent (ie., agonist, inverse agonist, or antagonist) under conditions effective to elicit a second messenger or other detectable responses upon interacting with the receptor molecule. The imidazoline receptive polypeptides are then contacted with one or more candidate chemical compounds (drugs), and any modification in a second messenger response is detected. Compounds that mimic an identified agonist would be agonist candidates, and those producing the opposite response would be inverse agonist candidates. Those compounds that block the effects of a known agonist would be

antagonist candidates for an in vivo imidazoline receptor. For meaningful results, the contacting step with a candidate compound is preferably conducted at a plurality of candidate compound concentrations.

A method of probing for another gene encoding an imidazoline receptor or homologous protein is further contemplated. Such a method comprises providing a radiolabelled DNA molecule identical or complementary to one of the above-described CDNA molecules (probe). The probe is then placed in contact with genetic material suspected of containing a gene encoding an imidazoline receptor or encoding a homologous protein, under stringent hybridization conditions (e.g., a high stringency wash condition is 0.1 x SSC, 0.5% SDS at 65°C), and identifying any portion of the genetic material that hybridizes to the DNA molecule.

Still further, a method of selectively producing antibodies, (e.g., monoclonal antibodies, immunoreactive with an instant imidazoline-receptive protein) comprises injecting a mammal with an aforementioned polypeptide, and isolating the antibodies produced by the mammal. This aspect is discussed in more detail in an example presented hereinafter.

The present inventors began their search for a human imidazoline receptor CDNA by screening a λgt11 phage human hippocampus CDNA expression library. Their research had indicated that both of the known antisera (Reis and Dontenwill) that are directed against human imidazoline receptors were immunoreactive with identical bands on SDS gels of membranes prepared from the human hippocampus (an in other

tissues). By contrast, other brain regions either were commercially unavailable as cDNA expression libraries or yielded inconsistencies between the two antisera. Therefore, it was felt that a human hippocampal cDNA library held the best opportunity for obtaining a CDNA for an imidazoline receptor. Immunoexpression screening was chosen over other cloning strategies because of its sensitivity when coupled with the ECL detection system used by the present inventors, as discussed hereinbelow.

A number of unique discoveries led to identifying the first 5A-1 clone as an imidazoline receptor CDNA. These included discoveries that led to the choice of a hippocampal CDNA library and adapting ECL to the antisera. Once the initial clone (5A-1) was identified and sequenced, a more complete clone (EST04033) was purchased without restriction from ATCC Inc. (Catalogue # 82815; American Type Culture Collection, Rockville, MD). EST 04033 was the only EST clone available at the time of the discovery of 5A-1, that contained a segment of complete homology (the origination of EST04033 is discussed later on). The binding affinities of the expressed protein after transfection in COS cells were determined by radioligand binding procedures developed in the inventor's laboratory [Piletz and Sletten, 1993, ibid].

To identify an instant CDNA clone encoding an imidazoline receptor it was preferable to employ both of the known antibodies to imidazoline receptors. These antibodies were obtained by contacting Dr. D. Reis (Cornell University Medical Center, New York City), and Drs. M. Dontenwill and P. Bousquet

(Laboratoire de Pharmacologie Cardiovascular et Renale, CNRS, Strasbourg, France). These antisera were obtained free of charge and without confidentiality or restrictions on their use. The former antiserum (Reis antiserum) was derived from a published imidazoline receptor protein [Wang et al., (1992, 1993), the disclosures of which are incorporated herein by reference]. The method for raising the latter antiserum (Dontenwill antiserum) has also been published [Bennai et al., (1995), the disclosure of which is also incorporated herein by reference]. The latter antiserum was developed using an anti-idiotypic approach that identified the pharmacologically correct (clonidine and idazoxan selective) binding site structure.

Example 1. Selectivity of the Antisera.

The obtained Reis antiserum had been prepared against a purified imidazoline binding protein isolated from BAC cells, which protein runs in denaturing-SDS gels at 70 Kda [Wang et al., 1992, 1993]. The Dontenwill antiserum is anti-idiotypic, and thus is believed to detect the molecular configuration of an imidazoline binding site domain in any species. Prior to being used for screening plaques, both antisera were cleaned by stripping out possible antibacterial antibodies.

Both antisera have been tested to ensure that they are in fact selective for a human imidazoline receptor. In particular, we found that both of these antisera detected identical bands in human platelets and hippocampus, and in brainstem RVLM (NRL) by Western blotting (see Fig. 1). In

these studies, in order to increase sensitivity over previously published detection methods, an ECL (Enhanced Chemiluminescence) system was employed. The linearity of response of the ECL system was demonstrated with a standard curve. ECL detection was demonstrated to be very quantifiable and about ten times more sensitive than other screening methods previously used with these antisera. Western blots with antiserum dilutions of 1:3000 revealed immunoreactivity with as little as 1 ng of protein from a human hippocampal homogenate by dot blot analysis.

For the studies depicted in Fig. 1, human hippocampal homogenate (30μg) and NRL membrane proteins (10μg) were electrophoresed through a 12.5% SDS-polyacrylamide gel, electrotransfered to nitrocellulose and sequentially incubated with (1) the Reis antibody (1:2000 dilution) and (2) the Dontenwill antibody (1:5000 dilution). Immunoreactive bands were visualized with an Enhanced Chemiluminescence (ECL) detection kit (Amersham) using anti-rabbit Ig-HRP conjugated antibody at a dilution of 1:3000 and the ECL detection reagents. Following detection with the antibody, blots were stripped and reprocessed omitting the primary antibody to check for complete removal of this antibody. In panels A and B, lane 1 shows the immunoreactive bands observed with the Reis antibody and lane 2 shows the bands detected with the Dontenwill antibody. Protein molecular weight standards are indicated to the left of each panel (in Kda).

Despite the diverse origins of the Reis and Dontenwill antisera, both of these antisera detected a similar 85 Kda

protein in human brain and other tissues. But, a 33 Kda band was found in human platelets. Although the 33 Kda band is of smaller size than that reported for other tissues [Wang et al., 1993; Escriba et al., 1994; Greney et al., 1994], the fact that both antisera detected it, suggests that both the 85 Kda and 33 Kda bands may be imidazoline binding polypeptides. The 85 and 33 Kda bands were enriched in plasma membrane fractions, as is known to be the case for IR_1 binding, but not I_2 binding [Piletz and Sletten, 1993].

A significant positive correlation was established for the 85 Kda band detected by the Dontenwill antiserum with IR_1 Bmax values across nine rat tissues ($r^2 = 0.8736$). A similar positive correlation was established amongst platelet samples from 15 healthy platelet donors between radioligand IR_1 Bmax values (but not I_2 or α_2AR Bmax values), and the 33 Kda band (presumed IR_1 immunoreactivity) on Western blots. This correlation exhibited a slope factor close to unity (results not shown). These correlations strongly suggested that an IR_1 binding protein might be revealed in an imidazoline receptor-antibody Western blotting assay. Furthermore, the Reis antiserum failed to detect authentic α_2AR, MAO A, or MAO B bands on gels, i.e., it was not immunoreactive with MAO at MW = 61 Kda, or α_2AR at MW = 64 Kda. Additionally, no immunoreactive bands were observed using preimmune antiserum. Thus, after extensively characterizing these antisera with human and rat materials, it was concluded that these antisera are indeed selective for human imidazoline receptor protein.

Example 2. Cloning of cDNA For An Imidazoline Receptor

A commercially available human hippocampal cDNA λgt11 expression library (Clontech Inc., Palo Alto, CA) was screened for immunoreactivity sequentially using both the anti-idiotypic Dontenwill antiserum and the Reis antiserum. Standard techniques to induce protein and transference to a nitrocellulose overlay were employed. [See, for instance, Sambrook et al., 1989, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press]. After washing and blocking with 5% milk, the Dontenwill antiserum was added to the overlay at 1:20,000 dilution in Tris-buffered saline, 0.05% Tween20, and 5% milk. The Reis antiserum was employed similarly, but at 1:15,000 dilution. These high dilutions of primary antiserum were chosen to avoid false positives. The secondary antibody was added, and positive plaques were identified using ECL. Representative results are shown in Fig. 2.

Positive plaques were pulled and rescreened until tertiary screenings yielded only positive plaques. Four separate positive plaques were identified from more than 300,000 primary plaques in our library. Recombinant λgt11 DNA purified from each of the four plaques was subsequently subcloned into E. coli pBluescript vector (Stratagene, La Jolla, CA). Sequencing of these four cDNA inserts in pBluescript demonstrated that they were identical, suggesting that only one cDNA had actually been identified four times. Thus, the screening had been verified as being highly reproducible and the frequency of occurrence was as expected

for a single copy gene, i.e., one in 75,000 transcripts. As shown in Fig. 2, the protein produced by the first positive clone, designated 5A-1, tested positive with both the Reis antiserum and the Dontenwill antiserum. Clone 5A-1 has been deposited under the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, USA, 20852, on August 28, 1997 and has been assigned deposit accession no. ATCC 209217. Tertiary-screened plaques of 5A-1 were all immuno-positive with either of the two known anti-imidazoline receptor antisera, but not with either preimmune antisera. These results suggested that clone 5A-1 encoded a fusion peptide similar to or identical with one of the predominant bands detected in human Western blots by both the Dontenwill and Reis antisera.

Sequencing of the first four clones was performed by contracting with ACGT Company (Chicago, IL) after subcloning them into pBluescript vector SK (Stratagene). Both manual and automatic sequencing strategies were employed which are outlined as follows:

Manual Sequencing

1. DNA sequencing was performed using T7 DNA polymerase and the dideoxy nucleotide termination reaction.

2. The primer walking method [Sambrook et al., ibid.] was used in both directions.

3. (^{35}S)dATP was used for labelling.

4. The reactions were analyzed on 6% polyacrylamide wedge or non-wedge gels containing 8 M urea, with samples being loaded in the order of A C G T.

5. DNA sequences were analyzed by MacVector Version 5.0. and by various Internet-available programs, i.e., the BLAST program.

Automatic Sequencing

1. DNA sequencing was performed by the fluorescent dye terminator labelling method using AmpliTaq DNA polymerase (Applied Biosystems Inc., Prizm DNA Sequencing Kit, Perkin-Elmer Corp., Foster City, CA).

2. The primer walking method was used. The primers actually used were a subset of those shown in SEQ ID Nos. 7-20.

3. Sequencing reactions were analyzed on an Applied Biosystems, Inc. (Foster City, CA) sequence analyzer.

These results demonstrated that the initial clone (5A-1) contained a 1171 base pair insert (see SEQ ID No. 4). The entire 5A-1 cDNA was found to exist as extended open reading frame for translation into protein. Consequently, it was determined that the 5A-1 cDNA must be a fragment of a larger mRNA.

cDNA Sequence Homologies

Using programs and databases available on the Internet (retrieved from NCBI Blast E-mail Server address blast@ncbi.nlm.nih.gov), it was determined that the 5A-1 clone encodes a previously undefined unique molecule. The BLASTP program [1.4.8MP, 20-June-1995 (build 11/13/95)] was used to compare all of the possible frames of amino acid sequences encoded by 5A-1 versus all known amino acid sequences

available within multiple international databases [Altschul et al., J. Mol. Biol., 215: 403-410 (1990)]. Only one protein, from *Micrococcus luteus*, possessed a marginally significant homology (p=0.04)(41%) over a short stretch of 75 of the 390 amino acids encoded by 5A-1. Otherwise, there were not any amino acid homologies (i.e., with $p \leq 0.05$) for any known proteins. Therefore, the protein encoded by 5A-1 is not significantly related to MAO A or B, α_2AR, or any other known eukaryotic protein in the literature.

In contrast to the amino acid search on BLASTP, two nearly homologous EST cDNA sequences of undefined nature covering 155 and 250 b.p. of the 5A-1 clone were reported to exist using BLASTN (reached from the same Internet server on 11/13/95). BLASTN is a program used to compare known DNA sequences from international databases, regardless of whether they encode a polypeptide. Neither of the two EST cDNA sequences having high homology to 5A-1, to our knowledge have been reported anywhere else except on the Internet. Both were derived as Expressed Sequence Tags (ESTs) in random attempts to sequence the human cDNA repertoire [as described in Adams et al., Science, 252: 1651-1656 (1991)]. As far as can be determined, the people who generated these ESTs lack any knowledge of what protein(s) they encode. One cDNA, designated HSA09H122, contained 250 b.p. with 7 unknown/incorrect base pairs (97% homology) versus 5A-1 over the same region. HSA09H122 was generated in France (Genethon, B.P. 60, 91002 Evry Cedex France) from a human lymphoblast cDNA library. The other EST, designated EST04033, contained

155 b.p. with 12 unknown/incorrect base pairs (92% homology) versus 5A-1 over the same region. EST04033 was generated at the Institute for Genomic Research (Gaithersburg, MD) from a human fetal brain cDNA clone (HFBDP28). Thus, both of these ESTs are short DNA sequences and contain a number of errors (typical of single-stranded sequencing procedures as used when randomly screening ESTs).

Based on the BLASTN search, the owner of HSA09H122 was contacted in an effort to obtain that clone. The current owner of the clone appears to be Dr. Charles Auffret (Paul Brousse Hospital, Genetique, B.P. 8, 94801 Villejuif Cedex, France). Dr. Auffret indicated by telephone that his clone came from a lot of clones believed to be contaminated with yeast DNA, and he did not trust it for release. Contamination with yeast DNA of that clone was later confirmed to have been reported within an Internet database. Thus, HSA09H122 was not reliable.

The other partial clone (EST04033) was purchased from American Type Culture Collection in Rockville, MD (ATCC Catalog no. 82815). A telephone call to the Institute for Genomic Research revealed that it had been deposited at ATCC under [insert terms]. As far as can be determined, the present inventors were the first to completely sequence EST04033. The complete size of EST04033 was 3389 b.p. (SEQ ID No. 1), with a 3,318 b.p. nonplasmid insert (see SEQ ID No. 3). Within this sequence of EST04033 the remaining 783 base pairs of the coding sequence presumed for a 70 kDa imidazoline receptor were predicted at the 5′ side of 5A-1 (i.e., 783 coding

nucleotides unique to EST04033 + 1171 coding nucleotides of 5A-1 = 1954 predicted total coding nucleotides; assuming b.p.# 1397-1400 in SEQ. No. 1 encodes the initiating methionine). The entire 1954 b.p. coding region for an ≈ 70 kDa protein is shown in SEQ ID No. 2. The nucleotide sequence of EST04033 was determined in the same manner as described previously for the 5A-1 clone. The nucleotide sequence of the entire clone is shown in SEQ ID No. 1. In this sequence, an identical overlap was observed for the sequence obtained previously for the 5A-1 clone and the sequence obtained for EST04033. The 5A-1 overlap began at EST04033 b.p. 2,181 (SEQ. No.1) and continued to the end of the molecule (b.p. 3,351).

Conclusions About Our cDNA Clones

cDNA of the present invention encode a protein that is immunoreactive with both of the known selective antisera for an imidazoline receptor, i.e., Reis antiserum and Dontenwill antiserum. Thus, an instant cDNA molecule produces a protein immunologically related to a purified imidazoline receptor and has the antigenic specificity expected for an imidazoline binding site. These antisera have been documented in the scientific literature as being selective for an "imidazoline receptor", which provides strong evidence that such an imidazoline receptor has indeed been cloned.

As mentioned, our instant cDNA sequence contains open reading frame distinct from any previously described proteins. Therefore, the encoded protein is novel, and it is unrelated to α_2-adrenoceptors or monoamine oxidases. Small hydrophobic

domains in the predicted amino acid sequence suggest that the protein is probably membrane bound, as expected for an imidazoline receptor.

Example 3. Cloning of a Human Gene

A pre-made genomic library of human placental DNA was purchased from Stratagene (La Jolla, CA) to screen for an IR gene by hybridization. The genomic library was constructed in Stratagene's vector λ FIX® II (catalog # 946206), and it was grown in XL1-Blue MRA (P2) host bacteria. It was titered to yield approximately 50,000 plaques per 137 mm plate. Lifts from six such plates were screened in duplicate by hybridization. The DNA probe used for screening was a 1.85 kb EcoR1 fragment from EST 04033 cDNA (uniquely related to our sequences based on the BLASTN). After the restriction digestion of EST 04033 DNA, the 1.85 kb fragment was extracted from an agarose electrophoresis gel, cleaned according to the GENECLEAN® III kit manual (BIO 101, Inc., P.O. Box 2284, La Jolla, CA), and radiolabeled with [α-^{32}P]d-CTP according to Stratagene's Prime-It® II Random Primer Labeling Kit manual. Plaques were lifted onto 137 mm Duralon-UV™ membranes (Stratagene's catalog #420102), denatured, and cross-linked with Stratgene's UV-Stratalinker™ 1800. Hybridization was conducted under high stringency conditions: prehybridization = 6 X SSC, 1 % SDS, 50 % formamide, and 100 1μg/ml of sheared, denatured salmon sperm DNA at 42°C for 2 hrs; hybridization = 6 X SSC, 1 % SDS, 50 % formamide, and 100 μg/ml of sheared, denatured salmon sperm DNA at 45°C overnight; wash = 2 washes

of 1 X SSC, 0.1 % SDS at 65°C and 3 washes of 0.2 X SSC, 0.2 % SDS at 65°C. This hybridization procedure is essentially described in Stratagene's vector λ FIX® II instruction manual. Positive plaques were localized by developing Kodak BioMax films. Two positive genomic clones of identical size were retained through three rounds of screening.

One of the positive genomic clones (designated JEP 1-A) was selected for complete characterization. It was found to contain an ≈ 17 kb insert. Large-scale preparations of this genomic clone DNA were performed using the λ QUICK! SPIN kit (BIO101, La Jolla, CA). To verify that we had cloned a gene corresponding to 5A-1 and EST04033 cDNA, some restriction site positions in the genomic clone were determined using the FLASH Nonradioactive Gene Mapping Kit (Stratagene) and compared to Southern blots of human DNA. The location of genomic sequences highly related to (or identical to) those of our cDNA clones was determined by high stringency hybridization (as above) with the following ^{32}P-labeled probe: a 1110 bp *Apa*I-*Eco*RI fragment from the cDNA clone 5A-1. This fragment was chosen as the probe because it lacks the GAG repeat (encoding glutamic acids), which might have complicated matters if it were found to be repeated elsewhere in the genome. With genomic clone JEP1-A, we detected a 14.1 kb *Eco*RI fragment and a 7.7 kb *Sac*I fragment that hybridized with this probe. Southern blots containing *Eco*RI- or *Sac*I-digested human genomic DNA (from human blood) with the 1110 bp *Apa*I-*Eco*RI cDNA probe also resulted in the detection of a 14.1 kb *Eco*RI fragment and a 7.7 kb *Sac*I fragment. No additional

restriction fragments of human genomic DNA appeared to hybridize with this probe under lower stringency conditions. These results strongly suggested that this gene (JEP-1A) encodes transcript(s) giving rise to the 5A-1 and EST04033 cDNA clones. Clone JEP-1A has been deposited under the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, USA, 20852, on August 28, 1997 and has been assigned deposit accession no. ATCC 209216.

Genomic DNA sequencing was done by contract with Cadus Pharmaceutical Corporation (Tarrytown, NY). The original lambda JEP1-A clone was subcloned into pZero (Invitrogen) as a convenient vector. The initial fragments for sequencing were derived from Sac I and Xba I restriction enzymes. The short Sac I fragments of 1.5, 3.0 and 3.5 kb were further digested with Hind III, Pst I, and Kpn I yielding 15 subclones of varying length. The procedure consisted of sequencing all these subclones and parent clones with vector forward and reverse primers. Subsequently, this initial round of sequencing was supplemented with primer walking using custom oligonucleotides. The Sac I fragments were joined together by primer walking using the 2 Xba I fragments of 3 and 10 Kb. Then, the largest Sac I fragment (8 kb) and the 10 kb Xba I fragment were used as templates for a transposon sequencing method. The method used was the Primer Island Transposition Kit (Perkin-Elmer Corp., Norwalk, CT; Applied Biosystems) (ABI). The kit consists of a synthetic transposon (Ty1) containing forward and reverse primers and the integrase

enzyme which inserts the transposon randomly into the target plasmid DNA. Transposon insertion is an alternative to subcloning or primer walking when sequencing a large region of DNA (Devine and Boeke, Nucleic Acids Res. 22: 3765-3772 (1994); Devine et al., Genome Res., in press, (1997); Kimmel et al., In Genome Analysis, a Laboratory Manual, Cold Spring Harbor Press, NY, NY, in press (1997). A total of over 250 individual sequencing reactions were performed. Sequencing was done on ABI model 373 and 377 automated sequencers using ABI dye-terminator sequencing kits. Primers were designed using Gene Runner software (Hastings Software, Hastings On Hudson, NY). Oligonucleotides were purchased from Gibco-BRL (Gaithersburg, MD). Sequence assembly was performed using Sequencer Software (Gene Codes Corp., Ann Arbor, MI) from 4-fold redundancy of sequences.

The entire sequence of our JEP-1A genomic clone is shown in SEQ. 21. The computer program, GENSCAN 1.0, was able to identify splice sites of known topology. As expected, this gene contained a number of introns. See Table 1 hereinbelow. Only one continuous open reading frame was identified within our genomic clone. This open reading frame was interrupted by a number of introns (which is typical of eukaryotic transcripts) as shown in Fig. 5. The predicted polypeptide is encoded by the genomic DNA beginning at b.p. # 971 of SEQ ID No. 21. The predicted amino acid sequence of the polypeptide encoded thereby is shown in SEQ ID No. 22. As can be seen, the entire 5A-1 DNA and polypeptide sequence was encapsulated within this predicted genomic transcript. Therefore, there is

no question that this is the gene encoding 5A-1 and EST04033 cDNA. In addition, JEP-1A has more nearly defined the full-length transcript (by at least 102 more coding nucleotides than the cDNAs alone).

TABLE 1

Position of Predicted Introns and Exons

GENSCAN 1.0 Date run: 26-Aug-97 Time: 12:35:39
Sequence gs_seqfile : 15202 bp : 58.36% C+G : Isochore 4 (57.00 - 100.00 C+G%)
Parameter matrix: HumanIso.smat
Predicted genes/exons:

Gn.Ex	Type	S	.Begin	...End	.Len	Fr	Ph	I/Ac	Do/T	CodRg	P..	Tscr..
1.01	Intr	+	971	1084	114	1	0	69	98	200	0.836	20.91
1.02	Intr	+	4096	4177	82	0	1	37	53	81	0.358	-0.13
1.03	Intr	+	5732	5856	125	0	2	117	95	84	0.953	13.48
1.04	Intr	+	6997	7046	50	0	2	95	116	44	0.998	6.52
1.05	Intr	+	8416	9825	1410	1	0	96	94	2914	0.970	283.09
1.06	Intr	+	10489	10897	409	1	1	15	59	318	0.517	17.19
1.07	Intr	+	11293	11449	157	0	1	57	61	236	0.998	18.57
1.08	Intr	+	11923	12051	129	2	0	84	63	224	0.997	21.34
1.09	Intr	+	12570	12731	162	1	0	95	80	229	0.996	23.94
1.10	Term	+	13090	13700	611	2	2	59	41	1012	0.942	89.44
1.11	PlyA	+	14257	14262	6							1.05

A BLASTN analysis of the entire genomic sequence (on 08/26/97) demonstrated again that this gene has not been previously defined in the literature.

As with the cDNA clones, some EST sequences of identity were found (listed below and later). Of particular interest was a variance in the first intron splice site predicted by the computer. Upstream of that site (ie., upstream of amino acids PEKKGGE = amino acids predicted after first splice site) we have identified two types of transcripts. Genomic clone JEP-1A predicted 34 amino acids upstream of that sequence before entering another intron upstream. In an identical

manner, three ESTs (H61282, AA428790 and AA428250) overlapped that entire region in our clones and they contained the identical nucleotides for those 34 amino acids, plus an additional 22 more amino acids further upstream. By comparison, however, our EST04033 varied from all of these ESTs upstream of that site. This means, the first 1,532 nucleotides of EST04033 (thought to encode translation of amino acids 1-56 of EST04033 beginning at b.p. 1,398 in SEQ. 1) are completely at variance with the other ESTs down to that splice site, but from there on they are identical. This provides strong evidence that this site can generate two alternatively spliced transcripts which can produce at least one functional protein (ie., the transfections with EST04033). For the reader's information, this splice site is upstream of b.p. # 1,565 in SEQ.1, b.p. # 168 in SEQ.2, b.p. # 1,532 in SEQ.3, amino acid # 57 in SEQ.5, and b.p. # 971 in the genomic SEQ.21.

Genomic Sequence Analysis

Of interest is a unique glutamic- and aspartic acid-rich region within our predicted protein. This region of the IR protein delineates a highly unique span of 59 amino acids, 36 of which are Glu or Asp residues (61%). This region was largely discovered within clone 5A-1 and it is present within all discovered and predicted transcripts from the gene (EST04033 included). This sequence lies between two potential transmembrane loops (hydrophobic domains). The identification of this unique Glu/Asp-rich domain within our

clones is consistent with an expected negatively charged pocket capable of binding clonidine and agmatine, both of which are highly positively charged ligands. Also, since the Dontenwill antiserum was specifically developed against an idazoxan/clonidine binding site, and its immunoreactivity is directed against the clone 5A-1/λgt11 fusion protein, this suggests that clone 5A-1 might encode an imidazoline binding site. Furthermore, this glu/asp-rich sequence is located within the longest stretch of homology that the clone has with any known protein, i.e., the ryanodine receptor (as determined by on BLASTN). Specifically, we have discovered four regions of homology between the imidazoline receptor and the ryanodine receptor, which are all Glu/Asp-rich. The total nucleic acid homology is 67% with the ryanodine receptor DNA over the stretches encompassing this region. However, this is not sufficient to indicate that the imidazoline receptor is a subtype of the ryanodine receptor, because this homologous stretch is still a minor portion of the overall transcript(s) identified in the gene. Instead, this significant homology may reflect a commonality in function between this region of the IR and the ryanodine receptor.

The Glu/Asp-rich region within the ryanodine receptor has also been reported to define a calcium and ruthenium red dye binding domain that modulates the ryanodine receptor/Ca^{++} release channel located within the sarcoplasmic reticulum. The only other charged amino acids within the Glu/Asp-rich region of our clones are two arginines (the ryanodine receptor has uncharged amino acids at the corresponding positions).

Based on this identification of Arg residues within the Glu/Asp-rich region of the predicted imidazoline binding site, the assistance of Dr. Paul Ernsberger (Case Western Reserve University, Cleveland, Ohio) was enlisted. Dr. Ernsberger performed phenylglyoxal attack of arginine on native PC-12 membranes. Dr. Ernsberger was able to demonstrate that this treatment completely eliminated imidazoline binding sites in these membranes. This provides some indirect evidence that the native imidazoline binding site also contains an Arg residue. On the other hand, attempts to chemically modify cysteine and tyrosine residues, which are not located near the Glu/Asp-rich region did not affect PC-12 membrane binding of imidazolines.

As a further test of the sequence, it was determined whether native IR binding sites in PC-12 cells would be sensitive to ruthenium red. From the structure of the cloned sequence, it was reasoned that native IR should bind ruthenium red. Accordingly, a competition of ruthenium red with ^{125}PIC at native IR sites on PC-12 membranes was studied. In these studies it was observed that ruthenium red competed for ^{125}PIC binding to the same extent as did the potent imidazoline compound, moxonidine, i.e., 100% competition. Furthermore, the IC_{50} for competition of ruthenium red at IR was slightly more robust than reported for ruthenium red on the activation of calcium-dependent cyclic nucleotide phosphodiesterase - the previous most potent effect of ruthenium red on any biological site - indicating possible pharmacological importance. It is also noteworthy that calcium failed to compete for ^{125}PIC

binding at PC-12 IR sites (as did a calcium substitute, lanthanum). We and others have previously reported that a number of other cations robustly interfere with IR binding [Ernsberger et al., Annals NY Acad.Sci., 763: 22-42 (1995); Ernsberger et al., Annals NY Acad.Sci., 763: 163-168 (1995)]. Attempts were also made to directly stain the proteins in SDS gels with ruthenium red [Chen and MacLennan, J. Biol. Chem., 269: 22698-22704 (1994)]. It was found that ruthenium red stains the same platelet (33 kDa) and brain (85 kDa) bands that Reis antiserum detects. (Remember, the same 33 kDa band was verified to directly correlate with ^{125}PIC Bmax values for IR.) Thus, these results linked the attributes predicted from the cloned sequence to a native IR binding site.

Two other facets of the predicted polypeptide from JEP-1A suggest that we have identified most of the functional sequences. First, our predicted protein is comparable in regard to both the order and size of three regions of importance to the function of the interleukin-2Rβ receptor (IL-2Rβ). Specifically, IL-2Rβ possesses the following regions over a span of 286 amino acids: ser-rich region, followed by glu/asp-rich region, followed by proline-rich region. Likewise, our predicted protein has the same three regions, in the same order, over a span of about 625 amino acids. This suggests that our protein might function similarly as cytokine receptors. Secondly, our predicted protein possesses a cytochrome p450 heme-iron ligand signature sequence [Nelson et al., Pharmacogenetics 6: 1-42 (1996)]. This suggests that our protein might also function as do

cytochrome p450s in oxidative, peroxidative and reductive metabolism of endogenous compounds.

Some additional findings about the amino acid sequence of our instant IR polypeptide are: (1) The glu/asp-rich region also bears similarity to an amino acid sequence within a GTPase activator protein. (2) There appear to be four small hydrophobic domains indicative of transmembrane domain receptors. (3) A number of potential protein kinase C (PKC) phosphorylation sites appear near to the carboxy side of the protein, and we have previously found that treatment of membranes with PKC leads to an enhancement of native IR binding. Thus, these observations are all consistent with other observations expected for native IR.

RNA Studies

Northern blotting has also been performed on polyA$^+$ mRNA from human tissues in order to ascertain the regional expression of the mRNA corresponding to our cDNA. The same 1110 b.p. *Apa*I-*Eco*RI fragment from cDNA clone 5A-1 used in Southern blots was used for these studies. This probe region was not found within any other known sequences on the BLASTN database. The results revealed a 6 kb mRNA band, which predominated over a much fainter 9.5 kb mRNA in most regions (Fig. 6). Some exceptions to this pattern were in lymph nodes and cerebellum (Fig. 6), where the 9.5 kb band was equally or more intense. Although the 6 kb band is weakly detectable in some non-CNS tissues, it is enriched in brain. An enrichment of the 6 kb mRNA is observed in brainstem, although not

exclusively. The regional distribution of the mRNA is somewhat in keeping with the reported distribution of IR binding sites, when extrapolated across species (Fig. 6). Thus, the rank order of Bmax values for IR in rat brain has been reported to be frontal cortex > hippocampus > medulla oblongata > cerebellum [Kamisaki et al., Brain Res., 514: 15-21 (1990)]. Therefore, with the exception of human cerebellum, which showed two mRNA bands, the distribution of the mRNA for our the present cloned cDNA is consistent with it belonging to IR.

[It should be noted that while IR binding sites are commonly considered to be low in cerebral cortex compared to brainstem, this is in fact a misinterpretation of the literature based only on comparisons to the alpha-2 adrenoceptor's Bmax, rather than on absolute values. Thus, IR Bmax values have actually been reported to be slightly higher in the cortex than the brainstem, but they only "appear" to be low in the cortex in comparison to the abundance of alpha-2 binding sites in cortex. Therefore, the distribution of the IR mRNA is reasonably in keeping with the actual Bmax values for radioligand binding to the receptor [Kamisaki et al., (1990)].

A final point to emphasize about the Northern blots is that they clearly demonstrate two high-stringency transcripts (Fig. 6). This is in keeping with the alternatively spliced EST cDNAs mentioned earlier. Thus, we suggest this may be the basis for both the 6 and 9.5 kb transcripts.

Summary of Genomic Sequence Results

The JEP-1A clone clearly contains most of the gene. Within it we have identified at least 3,776 nucleotides for transcript(s) (encoding 1,065 amino acids plus 587 b.p. of untranslated region down to the polyT$^+$ tail). This has been lengthened by at least 66 coding nucleotides upstream (22 amino acids) in comparison to overlapping ESTs. In addition to this, we are quite confident of the splice site for the two observed mRNA sizes. Most of the functional sequences are predicted to be encoded within our genomic clone.

A summary of the evidence that a gene encoding an imidazoline receptor protein has been cloned is summarized in Table 2 hereinbelow.

TABLE 2

Comparison of Protein Predicted From Our Clones with Properties of Native IR_1 and I_2 Sites

Imidazoline Receptor-like Clone	Authentic IR_1	Authentic I_2
Original λ phage fusion protein (from 5A-1) is immunoreactive with Dontenwill and Reis antibodies	Dontenwill-Ab activity (a)inhibits RVLM IR_1 binding (^{3}H-Clonidine), & (b) correlates with 85 kDa Western band. Reis-Ab activity correlates w platelet IR_1 Bmax (^{125}PIC binding)	Dontenwill & Reis Abs both inhibit brain I_2 sites (^{3}H-IDX).
Segment homologous to a GTPase-activator prot'n	Weak to moderate sensitivity to GTP	Not sensitive to GTP
Predicts ≥ 120,000 MW protein	85,000 MW immunoreactivity	59-61,000 MW photoaffinity
Predicts 1-4 hydrophobic domains	Enriched in plasma membranes	Enriched in mitochondria
Encodes Glu/Asp-rich (negatively charged) domain consistent with Ca^{++} and ruthenium red binding	• Binds (+)-charged imidazolines • Sensitive to divalent cations • Sensitive to ruthenium red	• Binds (+)-charged imidazolines • Not sensitive to divalent cations • Unknown sensitivity for Ruthen. red
Arginine is only positively charged amino acid near Glu/Asp domain	• Arg attack eliminates binding • Cys & Tyr attack w/o effect on binding	Unknown
Encodes PKC sites	PKC treatment enhances binding	Unknown
Human mRNA Distribution; F.Cortex > hippocampus > medulla	Rat IR_1 Bmax (^{125}PIC): F.Cortex > hippocampus > medulla	Rat I_2 Bmax (^{3}H-IDX): Medulla > F. Cortex
Transfected COS-7 cells expressed high affinity for moxonidine & p-iodoclonidine (PIC)	High affinity for moxonidine and PIC	Low affinity for moxonidine and PIC

Example 4. Transient Transfection Studies

COS-7 cells were transfected with a vector containing EST04033 cDNA, which was predicted based on sequence analysis to contain the glu/asp rich region thought to be important for ligand binding to the imidazoline receptor protein. The EST04033 cDNA was subcloned into pSVK3 (Pharmacia LKB Biotechnology, Piscataway, NJ) using standard techniques [Sambrook, supra], and transfected via the DEAE-dextran technique as previously described [Choudhary et al., Mol.Pharmacol., 42: 627-633 (1992); Choudhary et al., Mol.Pharmacol., 43: 557-561 (1993); Kohen et al., J.Neurochem., 66: 47-56 (1996)]. A restriction map of the EST04033 cDNA is shown in Fig. 3. The restriction enzymes Sal I and Xba I were used for subcloning into pSVK3.

Briefly stated, COS-7 cells were seeded at 3 x 10^6 cells/100 mm plate, grown overnight and exposed to 2 ml of DEAE-dextran/plasmid mixture. After a 10-15 min. exposure, 20 ml of complete medium (10% fetal calf serum; 5 μg/ml streptomycin; 100 units/ml penicillin, high glucose, Dulbeccos' modified Eagle's medium) containing 80 μM chloroquine was added and the incubation continued for 2.5 hr. at 37°C in a 5% CO_2 incubator. The mixture was then aspirated and 10 ml of complete medium containing 10% dimethyl sulfoxide was added with shaking for 150 seconds.

Following aspiration, 15 ml of complete medium with dialyzed serum was added and the incubation continued for an additional 65 hours. After this time period, the cells from 6 plates were harvested and membranes were prepared as

previously described [Ernsberger et al., Annals NY Acad. Sci., 763: 22-42 (1995), the disclosure of which is incorporated herein by reference]. Parent, untransfected COS-7 cells were prepared as a negative control. Some membranes were treated with and without PKC for 2 hrs prior to analysis, since previous studies had indicated that receptor phosphorylation could be beneficial to detect IR binding.

Transfected samples were also analyzed by Western blots. The protocol used for Western blot assay of transfected cells is as follows. Cell membranes were prepared in a special cocktail of protease inhibitors (1 mM EDTA, 0.1 mM EGTA, 1 mM phenylmethyl-sufonylfluoride, 10 mM ϵ-aminocaproic acid, 0.1 mM benzamide, 0.1 mM benzamide-HCl, 0.1 mM phenanthroline, 10 μg/ml pepstatin A, 5 mM iodoacetamide, 10 μg/ml antipain, 10 μg/ml trypsin-chymotrypsin inhibitor, 10 μg/ml leupeptin, and 1.67 μg/ml calpain inhibitor) in 0.25 M sucrose, 1 mM $MgCl_2$, 5 mM Tris, pH 7.4. Fifteen μg of total protein were denatured and separated by SDS gel electrophoresis. Gels were equilibrated and electrotransferred to nitrocellulose membranes. Blots were then blocked with 10% milk in Tris-buffered saline with 0.1% Tween-20 (TBST) during 60 min. of gentle rocking. Afterwards, blots were incubated in anti-imidazoline receptor antiserum (1:3000 dil.) for 2 hours. Following the primary antibody, blots were washed and incubated with horseradish peroxidase-conjugated anti-rabbit goat IgG (1:3000 dil.) for 1 hr. Blots were extensively washed and incubated for 1 min. in a 1:1 mix of Amersham ECL detection solution. The blots were wrapped in cling-film

(SARAN WRAP) and exposed to Hyperfilm-ECL (Amersham) for 2 minutes. Quantitation was based on densitometry using a standard curve of known amounts of protein containing BAC membranes or platelet membranes run in each gel.

One nM [^{125}I]p-iodoclonidine was employed in the radioligand binding competition assays, since at this low concentration this radioligand is selective for the IR site much more than for I_2 binding sites. The critical processes of membrane preparation of tissue culture cells and the radioligand binding assays of IR and I_2 have been reviewed by Piletz and colleagues [Ernsberger et al., Annals NY Acad Sci., 763: 510-519 (1995)]. Total binding (n = 12 per experiment) was determined in the absence of added competitive ligands and nonspecific binding was determined in the presence of 10^{-4} M moxonidine (n = 6 per experiment). Log normal competition curves were generated against unlabeled moxonidine, p-iodoclonidine, and (-) epinephrine. Each concentration of the competitors was determined in triplicate and the experiment was repeated thrice.

The protocol to fully characterize radioligand binding in the transfected cells entails the following. First, the presence of IR and/or I_2 binding sites are scanned over a range of protein concentrations using a single concentration of [^{125}I]-p-iodoclonidine (1.0nM) and ^{3}H-idazoxan (8nM), respectively. Then, rate of association binding experiments (under a 10 μM mask of NE to remove α_2AR interference) are performed to determine if the kinetic parameters are similar to those reported for native imidazoline receptors [Ernsberger

et al. Annals NY Acad. Sci., 763: 163-168 (1995)]. Then, full Scatchard plots of [^{125}I]-p-iodoclonidine (2-20 nM if like IR) and ^{3}H-idazoxan (5-60 nM if like I_2) binding are conducted under a 10 μM mask of NE. Total NE (10 μM)-displaceable binding is ascertained as a control to rule out α_2-adrenergic binding. The Bmax and K_D parameters for the transfected cells are ascertained by computer modeling using the LIGAND program [McPherson, G., J.Pharmacol.Meth., 14: 213-228 (1985)] using 20 μM moxonidine to define IR nonspecific binding, or 20 μM cirazoline to define I_2 nonspecific binding.

The results of the transient transfection experiments of the imidazoline receptor vector into COS-7 cells are shown in Fig. 4. Competition binding experiments were performed using membrane preparations from these cells and ^{125}PIC was used to radiolabel IR sites. A mask of 10 μM norepinephrine was used to rule out any possible α_2AR binding in each assay even though parent COS-7 cells lacked any α_2AR sites. Moxonidine and p-iodoclondine (PIC) were the compounds tested for their affinity to the membranes of transfected cells. As can be seen, the affinities of these compounds in competition with ^{125}PIC were well within the high affinity (nM) range.

The following IC_{50} values and Hill slopes were obtained in this study: moxonidine, IC_{50} = 45.1 nM (Hill slope = 0.35 ± 0.04); p-iodoclonidine without PKC pretreatment of the membranes, IC_{50} = 2.3 nM (Hill slope = 0.42 ± 0.06); p-iodoclonidine with PKC pretreatment of the membranes, IC_{50} = 19.0 nM (Hill slope = 0.48 ± 0.08). Shallow Hill slopes for [^{125}I]p-iodoclonidine have been reported before in studies of the interaction of moxonidine and p-iodoclonidine with the human platelet IR_1 binding site [Piletz

and Sletten, (1993)]. Epinephrine failed to displace any of the [^{125}I]p-iodoclonidine binding in the transfected cells, as expected since this is a nonadrenergic imidazoline receptor. Furthermore, in untransfected cells less than 5% of the amount of displaceable binding was observed as for the transfected cells - and this "noise" in the parent cells all appeared to be low affinity (data not shown). These results thus demonstrate the high affinities of two imidazoline compounds, p-iodoclonidine and moxonidine, for the portion of our cloned receptor encoded within EST04033. PKC pretreatment of the membranes had no effect in the transfected COS cells.

It was also observed that the level of the expressed protein, as measured by Western blotting of the transfected cells, was consistent with the level of IR binding that was detected. In other words, a protein band was uniquely detected in the transfected cells, and it was of a density consistent with the amount of radioligand binding. Hence, the present results are in keeping with those expected for an imidazoline receptor. In summary, these data provide direct evidence that the EST04033 clone encodes an imidazoline binding site having high affinities for moxonidine and p-iodoclonidine, which is expected for an IR protein.

Example 5. Stable Transfection Methods.

Stable transfections can be obtained by subcloning the imidazoline receptor cDNA into a suitable expression vector,

e.g., pRc/CMV (Invitrogen, San Diego, CA), which can then be used to transform host cells, e.g. CHO and HEK-293 cells, using the Lipofectin reagent (Gibco/BRL, Gaithersburg, MD) according to the manufacturer's instructions. These two host cell lines can be used to increase the permanence of expression of an instant clone. The inventors have previously ascertained that parent CHO cells lack both alpha$_2$-adrenoceptor and IR binding sites [Piletz et al., J. Pharm.& Exper. Ther., 272: 581-587 (1995)], making them useful for these studies. Twenty-four hours after transfection, cells are split into culture dishes and grown in the presence of 600 μg/ml G418-supplemented complete medium (Gibco/BRL). The medium is changed every 3 days and clones surviving in G418 are isolated and expanded for further investigation.

Example 6. Direct Cloning of More Complete Gene and Other Homologous Human IR.

Direct probing of other human genomic and cDNA libraries can be performed by preparing labelled cDNA probes from different subcloned regions of our clone. Commercially available human DNA libraries can be used. Besides the cDNA and genomic libraries we have already screened, another genomic library is EMBL (Clontech), which integrates genomic fragments up to 22 kbp long. It is reasonable to expect that introns may exist within other human IR genes so that only by obtaining overlapping clones can the full-length genes be sequenced. A probe encompassing the 5'

end of an instant cDNA is generally useful to obtain the gene promoter region. Clontech's Human PromoterFinder DNA Walking procedure provides a method for "walking" upstream or downstream from cloned sequences such as cDNAs into adjacent genomic DNA.

Example 7. Methods for Preparing Antibodies to Imidazoline Receptive Proteins.

An instant imidazoline receptive polypeptide can also be used to prepare antibodies immunoreactive therewith. Thus, synthetic peptides (based on deduced amino acid sequences from the DNA) can be generated and used as immunogens. Additionally, transfected cell lines or other manipulations of the DNA sequence of an instant imidazoline receptor can provide a source of purified imidazoline receptor peptides in sufficient quantities for immunization, which can lead to a source of selective antibodies having potential commercial value.

In addition, various kits for assaying imidazoline receptors can be developed that include either such antibodies or the purified imidazoline receptor protein. A purification protocol has already been published for the bovine imidazoline receptor in BAC cells [Wang et al, 1992] and an immunization protocol has also been published [Wang et al., 1993]. These same protocols can be utilized with little if any modification to afford purified human IR protein from transfected cells and to yield selective antibodies thereto.

In order to obtain antibodies to a subject peptide, the

peptide may be linked to a suitable soluble carrier to which antibodies are unlikely to be encountered in human serum. Illustrative carriers include bovine serum albumin, keyhole limpet hemocyanin, and the like. The conjugated peptide is injected into a mouse, or other suitable animal, where an immune response is elicited. Monoclonal antibodies can be obtained from hybridomas formed by fusing spleen cells harvested from the animal and myeloma cells [see, e.g., Kohler and Milstein, Nature, 256: 495-497 (1975)].

Once an antibody is prepared (either polyclonal or monoclonal), procedures are well established in the literature, using other proteins, to develop either RIA or ELISA assays [see, e.g., "Radioimmunoassay of Gut Regulatory Peptides; Methods in Laboratory Medicine," Vol. 2, chapters 1 and 2, Praeger Scientific Press, 1982]. In the case of RIA, the purified protein can also be radiolabelled and used as a radioactive antigen tracer.

Currently available methods to assay imidazoline receptors are unsuitable for routine clinical use, and therefore the development of an assay kit in this manner could have significant market appeal. Suitable assay techniques can employ polyclonal or monoclonal antibodies, as has been previously described [U.S. Patent No. 4,376,110 (issued to David et al.), the disclosure of which is incorporated herein by reference].

Summary

In summary, we have identified unique DNA sequences that have properties expected of a gene and the cDNA transcript(s) of an imidazoline receptor. Prior to our first cloning the cDNA, only two sequences of EST cDNA were identified within public databases having similar nature. But, these were both partial and imprecise sequences - not identified at all with respect to any encoded protein. Indeed, one of them (HSA09H122) was reported to be contaminated. In our hands, the other EST 04033 clone was correctly sequenced for the first time (in its entirety = 3318 bp). Prior to this, even the size of EST 04033 was unknown. The present inventors also demonstrated that an imidazoline receptive site can be expressed in cells transfected with the EST 04033 cDNA clone, and this site has the proper potencies of an IR. We have deduced most of the complete cDNA encoding this protein.

The present invention has been described with reference to specific examples for purposes of clarity and explanation. Certain obvious modifications of the invention readily apparent to one skilled in the art can be practiced within the scope of the appended claims.

SEQUENCE LISTING

GENERAL INFORMATION:

APPLICANT: John E. Piletz

Tina R. Ivanov

TITLE OF INVENTION: DNA MOLECULES ENCODING IMIDAZOLINE RECEPTIVE POLYPEPTIDES AND POLYPEPTIDES ENCODED THEREBY

NUMBER OF SEQUENCES: 22

CORRESPONDENCE ADDRESS:

ADDRESSEE: WENDEROTH, LIND & PONACK

STREET: 805 Fifteenth St. N.W., Suite 700

CITY: Washington

STATE: District of Columbia

COUNTRY: U.S.A.

ZIP: 20005

COMPUTER READABLE FORM:

MEDIUM TYPE: 3.50" 1.44 Mb diskette

COMPUTER: IBM PC compatible

OPERATING SYSTEM: MS-DOS

SOFTWARE: Wordperfect 5.1+

CURRENT APPLICATION DATA:

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FILING DATE: May 20, 1996

ATTORNEY/AGENT INFORMATION:

NAME: Warren Cheek

REGISTRATION NUMBER: 33,367

REFERENCE/DOCKET NUMBER: WMC-1342/clone

TELECOMMUNICATION INFORMATION:

TELEPHONE: (202) 371-8850

TELEFAX: (202) 371-8856

INFORMATION FOR SEQ ID NO: 1

SEQUENCE CHARACTERISTICS:

LENGTH: 3389 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

IMMEDIATE SOURCE:

LIBRARY: cDNA

CLONE: EST04033 (HFBDP28)

FEATURE:

NAME/KEY: predicted translation product when transfected

LOCATION: 1398 ... 3389

SEQUENCE DESCRIPTION: SEQ ID NO: 1

GCTCTAGAAC TAGTGGATCC CCCGGGCTGC AGGAATTCCA GTTTAATACT AACCCTAATG 60

TGTGACTGCG GTTTACAAAG AGCTCTGTAT CACCTGGGAT AGCTTTCAGT AGCAATTCAC 120

TACAACTGGT CCTAAAAAAT AATAACAATA ATAATAATAA TTAGAGAATT AAAACCCAAC 180

AGCATGTTGA ATGGTTAAAA TCACGTAAGA ACTGAAATTT GGGGTGGGGG TGTCCTCAAC 240

AGCTGAGCTT GTCCTAGCAG TGAAAATGCT CGCCTCCAAG CAGGGCTCAG AAAGGTCTGG 300

AGCCCTCCAG GCAGAGGGCT GAGCTCAGGG GGCTCTTGGA GGACACTCAC CCCATGGTCC 360

ATGGGATGCT TCTGGCTTCC TTAAAAACAG TTGGGCATCC GCATTGTATA AGTAGGTGGA 420

GACCCTAGTG TGGTTCTTTT GAAGGATATG GGAAGGGAGG ATGACGAACT AGAGAAGTGG 480

GAGGGGACCA AAATCACTGA GGTCCCAGAA TATCATAGAT TTGGGTATAG GATTGGGGTC 540

ACTAAGAATT GAGCACCAGG AATTCCAGCT TCTTCCCATT AAAGAAACTG GGACTGGTTT 600

TGCCTTGGAG GCCTATGTAG TGTTTTCTGC CCCTGTCCCA TACCAAGTCT CATTGATATT 660

TCTGCAGAAT ATCAGATGAA AATCTATTTC TAAAGACCAT TGGGAGAATG GGTGGTGGAG 720

AAGGAGTTGG AGTGGGGTTG GGGGGCAGTT AAAAATGAAT AAAAATCTCT CAGCTACAGA 780

ACCCAAACAT CACTTCCCTC CGCATTCACA GCATTTCCCA GCAGTCCCCA GATGGTTGTT 840

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TCCGTGGGGA CACAGCAGCT GCCTCATTTC CCTTCAGGCC CCATGGGCTG CTGGTCAACC   900
TCAGGATCTA CTAAAGATGA CGCAAATGCC GACTGAACAA TCTGAAACCC AAAGGACTCG   960
AGGAGAGACA TGTTCTGCTG AGGAGAGAAA GGTGAGCCAA GGGCAGGGCC CAGGTCCCCC  1020
AGGGGGCCCC CGAGAGCCCG GACATGCACC TTCTGGATGT GTTTGTTCAA GTAGGACTTA  1080
GAGCGGAAGA AGCTCCCACA TTCAGGGCAT GGGTACTTCT TCTCCCCATC AGACTCCATT  1140
TTGTTTTTGG GGACTGCCAT GTCGCAGGAG AAAGAGCCAT TGGCACTCTG CTTCTCTGGC  1200
GTCTTCAGGT CGCTGGCATC TGAGAGGTCA CCATAGGAGT CAGAGCTCTC AATCGGATCC  1260
TGATGTGAGC ATTTCTGGCC TTCTCGGTTA CAGATACTGC AGAAGTTGCT GGGCCCCTCG  1320
CTGTGCTTCT TCAGGTGGTC TGCCATGTAT GCTGCCCGCA AGTACTTCCC ACACACCTGG  1380
CAGGGCACCT TGTCTTC  ATG ACA GGC CAG GTG GGA GCG CAG ACG GTC TCG     1430
                   Met Thr Gly Gln Val Gly Ala Gln Thr Val Ser
                   1               5                  10

GGT GGC AAA AGA AGC ATT GCA GGT CTG ACA CTT GTG AGG CCG CTC AGA     1478
Gly Gly Lys Arg Ser Ile Ala Gly Leu Thr Leu Val Arg Pro Leu Arg
            15                  20                  25

AGT GTG CAC CTG CTT GAT ATG TCC GTT CAA GTG ATC AGG CCT GGA GAA     1526
Ser Val His Leu Leu Asp Met Ser Val Gln Val Ile Arg Pro Gly Glu
        30                  35                  40

GCC TTT CCC ACA GCT CTG GCA GAT GTA AGG CGG AAT TCC CCA GAG AAG     1574
Ala Phe Pro Thr Ala Leu Ala Asp Val Arg Trp Asn Ser Pro Glu Lys
    45                  50                  55

AAG GGT GGT GAA GAC TCC CGG CTC TCA GCT GCC CCC TGC ATC AGA CCC     1622
Lys Gly Gly Glu Asp Ser Trp Leu Ser Ala Ala Pro Cys Ile Arg Pro
60                  65                  70                  75

AGC AGC TCC CCT CCC ACT GTG GCT CCC GCA TCT GCC TCC CTG CCC CAG     1670
Ser Ser Ser Pro Pro Thr Val Ala Pro Ala Ser Ala Ser Leu Pro Gln
                80                  85                  90

CCC ATC CTC TCT AAC CAA GGA ATC ATG TTC GTT CAG GAG GAG GCC CTG     1718
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Pro Ile Leu Ser Asn Gln Gly Ile Met Phe Val Gln Glu Glu Ala Leu
            95                  100                 105

GCC AGC AGC CTC TCG TCC ACT GAC AGT CTG ACT CCC GAG CAC CAG CCC    1766
Ala Ser Ser Leu Ser Ser Thr Asp Ser Leu Thr Pro Glu His Gln Pro
        110                 115                 120

ATT GCC CAG GGA TGT TCT GAT TCC TTG GAG TCC ATC CCT GCG GGA CAG    1814
Ile Ala Gln Gly Cys Ser Asp Ser Leu Glu Ser Ile Pro Ala Gly Gln
    125                 130                 135

GCA GCT TCC GAT GAT TTA AGG GAC GTG CCA GGA GCT GTT GGT GGT GCA    1862
Ala Ala Ser Asp Asp Leu Arg Asp Val Pro Gly Ala Val Gly Gly Ala
140                 145                 150                 155

AGC CCA GAA CAT GCC GAG CCG GAG GTC CAG GTG GTG CCG GGG TCT GGC    1910
Ser Pro Glu His Ala Glu Pro Glu Val Gln Val Val Pro Gly Ser Gly
                160                 165                 170

CAG ATC ATC TTC CTG CCC TTC ACC TGC ATT GGC TAC ACG GCC ACC AAT    1958
Gln Ile Ile Phe Leu Pro Phe Thr Cys Ile Gly Tyr Thr Ala Thr Asn
            175                 180                 185

CAG GAC TTC ATC CAG CGC CTG AGC ACA CTG ATC CGG CAG GCC ATC GAG    2006
Gln Asp Phe Ile Gln Arg Leu Ser Thr Leu Ile Trp Gln Ala Ile Glu
        190                 195                 200

CGG CAG CTG CCT GCC TGG ATC GAG GCT GCC AAC CAG CGG GAG GAG GGC    2054
Trp Gln Leu Pro Ala Trp Ile Glu Ala Ala Asn Gln Trp Glu Glu Gly
    205                 210                 215

CAG GGT GAA CAG GGC GAG GAG GAG GAT GAG GAG GAG GAA GAA GAG GAG    2102
Gln Gly Glu Gln Gly Glu Glu Glu Asp Glu Glu Glu Glu Glu Glu Glu
220                 225                 230                 235
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GAC GTG GCT GAG AAC CGC TAC TTT GAA ATG GGG CCC CCA GAC GTG GAG     2150
Asp Val Ala Glu Asn Arg Tyr Phe Glu Met Gly Pro Pro Asp Val Glu
                240                 245                 250

GAG GAG GAG GGA GGA GGC CAG GGG GAG GAA GAG GAG GAG GAA GAG GAG     2198
Glu Glu Glu Gly Gly Gly Gln Gly Glu Glu Glu Glu Glu Glu Glu Glu
            255                 260                 265

GAT GAA GAG GCC GAG GAG GAG CGC CTG GCT CTG GAA TGG GCC CTG GGC     2246
Asp Glu Glu Ala Glu Glu Glu Arg Leu Ala Leu Glu Trp Ala Leu Gly
        270                 275                 280

GCG GAC GAG GAC TTC CTG CTG GAG CAC ATC CGC ATC CTC AAG GTG CTG     2294
Ala Asp Glu Asp Phe Leu Leu Glu His Ile Arg Ile Leu Lys Val Leu
    285                 290                 295

TGG TGC TTC CTG ATC CAT GTG CAG GGC AGT ATC CGC CAG TTC GCC GCC     2342
Trp Cys Phe Leu Ile His Val Gln Gly Ser Ile Arg Gln Phe Ala Ala
300                 305                 310                 315

TGC CTT GTG CTC ACC GAC TTC GGC ATC GCA GTC TTC GAG ATC CCG CAC     2390
Cys Leu Val Leu Thr Asp Phe Gly Ile Ala Val Phe Glu Ile Pro His
                320                 325                 330

CAG GAG TCT CGG GGC AGC AGC CAG CAC ATC CTC TCC TCC CTG CGC TTT     2438
Gln Glu Ser Trp Gly Ser Ser Gln His Ile Leu Ser Ser Leu Arg Phe
            335                 340                 345

GTC TTT TGC TTC CCG CAT GGC GAC CTC ACC GAG TTT GGC TTC CTC ATG     2486
Val Phe Cys Phe Pro His Gly Asp Leu Thr Glu Phe Gly Phe Leu Met
        350                 355                 360

CCG GAG CTG TGT CTG GTG CTC AAG GTA CGG CAC AGT GAG AAC ACG CTC     2534
Pro Glu Leu Cys Leu Val Leu Lys Val Arg His Ser Glu Asn Thr Leu
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365 370 375

TTC ATT ATC TCG GAC GCC GCC AAC CTG CAC GAG TTC CAC GCG GAC CTG 2582
Phe Ile Ile Ser Asp Ala Ala Asn Leu His Glu Phe His Ala Asp Leu
380 385 390 395

CGC TCA TGC TTT GCA CCC CAG CAC ATG GCC ATG CTG TGT AGC CCC ATC 2630
Arg Ser Cys Phe Ala Pro Gln His Met Ala Met Leu Cys Ser Pro Ile
400 405 410

CTC TAC GGC AGC CAC ACC AGC CTG CAG GAG TTC CTG CGC CAG CTG CTC 2678
Leu Tyr Gly Ser His Thr Ser Leu Gln Glu Phe Leu Arg Gln Leu Leu
415 420 425

ACC TTC TAC AAG GTG GCT GGC GGC TGC CAG GAG CGC AGC CAG GGC TGC 2726
Thr Phe Tyr Lys Val Ala Gly Gly Cys Gln Glu Arg Ser Gln Gly Cys
430 435 440

TTC CCC GTC TAC CTG GTC TAC AGT GAC AAG CGC ATG GTG CAG ACG GCC 2774
Phe Pro Val Tyr Leu Val Tyr Ser Asp Lys Arg Met Val Gln Thr Ala
445 450 455

GCC GGG GAC TAC TCA GGC AAC ATC GAG TGG GCC AGC TGC ACA CTC TGT 2822
Ala Gly Asp Tyr Ser Gly Asn Ile Glu Trp Ala Ser Cys Thr Leu Cys
460 465 470 475

TCA GCC GTG CGG CGC TCC TGC TGC GCG CCC TCT GAG GCC GTC AAG TCC 2870
Ser Ala Val Arg Arg Ser Cys Cys Ala Pro Ser Glu Ala Val Lys Ser
480 485 490

GCC GCC ATC CCC TAC TGG CTG TTG CTC ACG CCC CAG CAC CTC AAC GTC 2918
Ala Ala Ile Pro Tyr Trp Leu Leu Leu Thr Pro Gln His Leu Asn Val
495 500 505

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ATC AAG GCC GAC TTC AAC CCC ATG CCC AAC CGT GGC ACC CAC AAC TGT     2966
Ile Lys Ala Asp Phe Asn Pro Met Pro Asn Arg Gly Thr His Asn Cys
        510                 515                 520

CGC AAC CGC AAC AGC TTC AAG CTC AGC CGT GTG CCG CTC TCC ACC GTG     3014
Arg Asn Arg Asn Ser PHe Lys Leu Ser Arg Val Pro Leu Ser Thr Val
    525                 530                 535

CTG CTG GAC CCC ACA CGC AGC TGT ACC CAG CCT CGG GGC GCC TTT GCT     3062
Leu Leu Asp Pro Thr Arg Ser Cys Thr Gln Pro Arg Gly Ala Phe Ala
540                 545                 550                 555

GAT GGC CAC GTG CTA GAG CTG CTC GTG GGG TAC CGC TTT GTC ACT GCC     3110
Asp Gly His Val Leu Glu Leu Leu Val Gly Tyr Arg Phe Val Thr Ala
                560                 565                 570

ATC TTC GTG CTG CCC CAC GAG AAG TTC CAC TTC CTG CGC GTC TAC AAC     3158
Ile Phe Val Leu Pro His Glu Lys Phe His Phe Leu Arg Val Tyr Asn
            575                 580                 585

CAG CTG CGG GCC TCG CTG CAG GAC CTG AAG ACT GTG GTC ATC GCC AAG     3206
Gln Leu Arg Ala Ser Leu Gln Asp Leu Lys Thr Val Val Ile Ala Lys
        590                 595                 600

ACC CCC GGG ACG GGA GGC AGC CCC CAG GGC TCC TTT GCG GAT GGC CAG     3254
Thr Pro Gly Thr Gly Gly Ser Pro Gln Gly Ser Phe Ala Asp Gly Gln
    605                 610                 615

CCT GCC GAG CGC AGG GCC AGC AAT GAC CAG CGT CCC CAG GAG GTC CCA     3302
Pro Ala Glu Arg Arg Ala Ser Asn Asp Gln Arg Pro Gln Glu Val Pro
620                 625                 630                 635

GCA GAG GCT CTG GCC CCG GCC CCA GTG GAA GTC CCA GCT CCA GCC CCG     3350
Ala Glu Ala Leu Ala Pro Ala Pro Val Glu Val Pro Ala Pro Ala Pro
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640 645 650

GAA TTC GAT ATC AAG CTT ATC GAT ACC GTC GAC CTG CAG 3389
Glu Phe Asp Ile Lys Leu Ile Asp Thr Val Asp Leu Gln
655 660 664

INFORMATION FOR SEQ ID NO: 2

SEQUENCE CHARACTERISTICS:

LENGTH: 1954 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 2

ATGACAGGCC AGGTGGGAGC GCAGACGGTC TCGGGTGGCA AAAGAAGCAT TGCAGGTCTG 60
ACACTTGTGA GGCCGCTCAG AAGTGTGCAC CTGCTTGATA TGTCCGTTCA AGTGATCAGG 120
CCTGGAGAAG CCTTTCCCAC AGCTCTGGCA GATGTAAGGC GGAATTCCCC AGAGAAGAAG 180
GGTGGTGAAG ACTCCCGGCT CTCAGCTGCC CCCTGCATCA GACCCAGCAG CTCCCCTCCC 240
ACTGTGGCTC CCGCATCTGC CTCCCTGCCC CAGCCCATCC TCTCTAACCA AGGAATCATG 300
TTCGTTCAGG AGGAGGCCCT GGCCAGCAGC CTCTCGTCCA CTGACAGTCT GACTCCCGAG 360
CACCAGCCCA TTGCCCAGGG ATGTTCTGAT TCCTTGGAGT CCATCCCTGC GGGACAGGCA 420
GCTTCCGATG ATTTAAGGGA CGTGCCAGGA GCTGTTGGTG GTGCAAGCCC AGAACATGCC 480
GAGCCGGAGG TCCAGGTGGT GCCGGGGTCT GGCCAGATCA TCTTCCTGCC CTTCACCTGC 540
ATTGGCTACA CGGCCACCAA TCAGGACTTC ATCCAGCGCC TGAGCACACT GATCCGGCAG 600
GCCATCGAGC GGCAGCTGCC TGCCTGGATC GAGGCTGCCA ACCAGCGGGA GGAGGGCCAG 660
GGTGAACAGG GCGAGGAGGA GGATGAGGAG GAGGAAGAAG AGGAGGACGT GGCTGAGAAC 720
CGCTACTTTG AAATGGGGCC CCCAGACGTG GAGGAGGAGG AGGGAGGAGG CCAGGGGGAG 780
GAAGAGGAGG AGGAAGAGGA GGATGAAGAG GCCGAGGAGG AGCGCCTGGC TCTGGAATGG 840
GCCCTGGGCG CGGACGAGGA CTTCCTGCTG GAGCACATCC GCATCCTCAA GGTGCTGTGG 900
TGCTTCCTGA TCCATGTGCA GGGCAGTATC CGCCAGTTCG CCGCCTGCCT TGTGCTCACC 960
GACTTCGGCA TCGCAGTCTT CGAGATCCCG CACCAGGAGT CTCGGGGCAG CAGCCAGCAC 1020
ATCCTCTCCT CCCTGCGCTT TGTCTTTTGC TTCCCGCATG GCGACCTCAC CGAGTTTGGC 1080
TTCCTCATGC CGGAGCTGTG TCTGGTGCTC AAGGTACGGC ACAGTGAGAA CACGCTCTTC 1140
ATTATCTCGG ACGCCGCCAA CCTGCACGAG TTCCACGCGG ACCTGCGCTC ATGCTTTGCA 1200

CCCCAGCACA TGGCCATGCT GTGTAGCCCC ATCCTCTACG GCAGCCACAC CAGCCTGCAG 1260

GAGTTCCTGC GCCAGCTGCT CACCTTCTAC AAGGTGGCTG GCGGCTGCCA GGAGCGCAGC 1320

CAGGGCTGCT TCCCCGTCTA CCTGGTCTAC AGTGACAAGC GCATGGTGCA GACGGCCGCC 1380

GGGGACTACT CAGGCAACAT CGAGTGGGCC AGCTGCACAC TCTGTTCAGC CGTGCGGCGC 1440

TCCTGCTGCG CGCCCTCTGA GGCCGTCAAG TCCGCCGCCA TCCCCTACTG GCTGTTGCTC 1500

ACGCCCCAGC ACCTCAACGT CATCAAGGCC GACTTCAACC CCATGCCCAA CCGTGGCACC 1560

CACAACTGTC GCAACCGCAA CAGCTTCAAG CTCAGCCGTG TGCCGCTCTC CACCGTGCTG 1620

CTGGACCCCA CACGCAGCTG TACCCAGCCT CGGGGCGCCT TTGCTGATGG CCACGTGCTA 1680

GAGCTGCTCG TGGGGTACCG CTTTGTCACT GCCATCTTCG TGCTGCCCCA CGAGAAGTTC 1740

CACTTCCTGC GCGTCTACAA CCAGCTGCGG GCCTCGCTGC AGGACCTGAA GACTGTGGTC 1800

ATCGCCAAGA CCCCCGGGAC GGGAGGCAGC CCCCAGGGCT CCTTTGCGGA TGGCCAGCCT 1860

GCCGAGCGCA GGGCCAGCAA TGACCAGCGT CCCCAGGAGG TCCCAGCAGA GGCTCTGGCC 1920

CCGGCCCCAG TGGAAGTCCC AGCTCCAGCC CCGG 1954

INFORMATION FOR SEQ ID NO: 3

SEQUENCE CHARACTERISTICS:

LENGTH: 3318 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 3

AATTCCAGTT TAATACTAAC CCTAATGTGT GACTGCGGTT TACAAAGAGC TCTGTATCAC 60

CTGGGATAGC TTTCAGTAGC AATTCACTAC AACTGGTCCT AAAAAATAAT AACAATAATA 120

ATAATAATTA GAGAATTAAA ACCCAACAGC ATGTTGAATG GTTAAAATCA CGTAAGAACT 180

GAAATTTGGG GTGGGGGTGT CCTCAACAGC TGAGCTTGTC CTAGCAGTGA AAATGCTCGC 240

CTCCAAGCAG GGCTCAGAAA GGTCTGGAGC CCTCCAGGCA GAGGGCTGAG CTCAGGGGGC 300

TCTTGGAGGA CACTCACCCC ATGGTCCATG GGATGCTTCT GGCTTCCTTA AAAACAGTTG 360

GGCATCCGCA TTGTATAAGT AGGTGGAGAC CCTAGTGTGG TTCTTTTGAA GGATATGGGA 420

AGGGAGGATG ACGAACTAGA GAAGTGGGAG GGGACCAAAA TCACTGAGGT CCCAGAATAT 480

CATAGATTTG GGTATAGGAT TGGGGTCACT AAGAATTGAG CACCAGGAAT TCCAGCTTCT 540

TCCCATTAAA GAAACTGGGA CTGGTTTTGC CTTGGAGGCC TATGTAGTGT TTTCTGCCCC 600

TGTCCCATAC CAAGTCTCAT TGATATTTCT GCAGAATATC AGATGAAAAT CTATTTCTAA 660

AGACCATTGG GAGAATGGGT GGTGGAGAAG GAGTTGGAGT GGGGTTGGGG GGCAGTTAAA 720

AATGAATAAA AATCTCTCAG CTACAGAACC CAAACATCAC TTCCCTCCGC ATTCACAGCA 780

TTTCCCAGCA GTCCCCAGAT GGTTGTTTCC GTGGGGACAC AGCAGCTGCC TCATTTCCCT 840

TCAGGCCCCA TGGGCTGCTG GTCAACCTCA GGATCTACTA AAGATGACGC AAATGCCGAC 900

TGAACAATCT GAAACCCAAA GGACTCGAGG AGAGACATGT TCTGCTGAGG AGAGAAAGGT 960

GAGCCAAGGG CAGGGCCCAG GTCCCCCAGG GGGCCCCCGA GAGCCCGGAC ATGCACCTTC 1020

TGGATGTGTT TGTTCAAGTA GGACTTAGAG CGGAAGAAGC TCCCACATTC AGGGCATGGG 1080

TACTTCTTCT CCCCATCAGA CTCCATTTTG TTTTTGGGGA CTGCCATGTC GCAGGAGAAA 1140

GAGCCATTGG CACTCTGCTT CTCTGGCGTC TTCAGGTCGC TGGCATCTGA GAGGTCACCA 1200

TAGGAGTCAG AGCTCTCAAT CGGATCCTGA TGTGAGCATT TCTGGCCTTC TCGGTTACAG 1260

ATACTGCAGA AGTTGCTGGG CCCCTCGCTG TGCTTCTTCA GGTGGTCTGC CATGTATGCT 1320

GCCCGCAAGT ACTTCCCACA CACCTGGCAG GGCACCTTGT CTTCATGACA GGCCAGGTGG 1380

GAGCGCAGAC GGTCTCGGGT GGCAAAAGAA GCATTGCAGG TCTGACACTT GTGAGGCCGC 1440

TCAGAAGTGT GCACCTGCTT GATATGTCCG TTCAAGTGAT CAGGCCTGGA GAAGCCTTTC 1500

CCACAGCTCT GGCAGATGTA AGGCGGAATT CCCCAGAGAA GAAGGGTGGT GAAGACTCCC 1560

GGCTCTCAGC TGCCCCCTGC ATCAGACCCA GCAGCTCCCC TCCCACTGTG GCTCCCGCAT 1620

CTGCCTCCCT GCCCCAGCCC ATCCTCTCTA ACCAAGGAAT CATGTTCGTT CAGGAGGAGG 1680

CCCTGGCCAG CAGCCTCTCG TCCACTGACA GTCTGACTCC CGAGCACCAG CCCATTGCCC 1740

AGGGATGTTC TGATTCCTTG GAGTCCATCC CTGCGGGACA GGCAGCTTCC GATGATTTAA 1800

GGGACGTGCC AGGAGCTGTT GGTGGTGCAA GCCCAGAACA TGCCGAGCCG GAGGTCCAGG 1860

TGGTGCCGGG GTCTGGCCAG ATCATCTTCC TGCCCTTCAC CTGCATTGGC TACACGGCCA 1920

CCAATCAGGA CTTCATCCAG CGCCTGAGCA CACTGATCCG GCAGGCCATC GAGCGGCAGC 1980

TGCCTGCCTG GATCGAGGCT GCCAACCAGC GGGAGGAGGG CCAGGGTGAA CAGGGCGAGG 2040

AGGAGGATGA GGAGGAGGAA GAAGAGGAGG ACGTGGCTGA GAACCGCTAC TTTGAAATGG 2100

GGCCCCCAGA CGTGGAGGAG GAGGAGGGAG GAGGCCAGGG GGAGGAAGAG GAGGAGGAAG 2160

AGGAGGATGA AGAGGCCGAG GAGGAGCGCC TGGCTCTGGA ATGGGCCCTG GGCGCGGACG 2220

AGGACTTCCT GCTGGAGCAC ATCCGCATCC TCAAGGTGCT GTGGTGCTTC CTGATCCATG 2280

TGCAGGGCAG TATCCGCCAG TTCGCCGCCT GCCTTGTGCT CACCGACTTC GGCATCGCAG 2340

TCTTCGAGAT CCCGCACCAG GAGTCTCGGG GCAGCAGCCA GCACATCCTC TCCTCCCTGC 2400

GCTTTGTCTT TTGCTTCCCG CATGGCGACC TCACCGAGTT TGGCTTCCTC ATGCCGGAGC 2460

TGTGTCTGGT GCTCAAGGTA CGGCACAGTG AGAACACGCT CTTCATTATC TCGGACGCCG 2520

CCAACCTGCA CGAGTTCCAC GCGGACCTGC GCTCATGCTT TGCACCCCAG CACATGGCCA 2580

TGCTGTGTAG CCCCATCCTC TACGGCAGCC ACACCAGCCT GCAGGAGTTC CTGCGCCAGC 2640

TGCTCACCTT CTACAAGGTG GCTGGCGGCT GCCAGGAGCG CAGCCAGGGC TGCTTCCCCG 2700

TCTACCTGGT CTACAGTGAC AAGCGCATGG TGCAGACGGC CGCCGGGGAC TACTCAGGCA 2760

ACATCGAGTG GGCCAGCTGC ACACTCTGTT CAGCCGTGCG GCGCTCCTGC TGCGCGCCCT 2820

CTGAGGCCGT CAAGTCCGCC GCCATCCCCT ACTGGCTGTT GCTCACGCCC CAGCACCTCA 2880

ACGTCATCAA GGCCGACTTC AACCCCATGC CCAACCGTGG CACCCACAAC TGTCGCAACC 2940

GCAACAGCTT CAAGCTCAGC CGTGTGCCGC TCTCCACCGT GCTGCTGGAC CCCACACGCA 3000

GCTGTACCCA GCCTCGGGGC GCCTTTGCTG ATGGCCACGT GCTAGAGCTG CTCGTGGGGT 3060

ACCGCTTTGT CACTGCCATC TTCGTGCTGC CCCACGAGAA GTTCCACTTC CTGCGCGTCT 3120

ACAACCAGCT GCGGGCCTCG CTGCAGGACC TGAAGACTGT GGTCATCGCC AAGACCCCCG 3180

GGACGGGAGG CAGCCCCCAG GGCTCCTTTG CGGATGGCCA GCCTGCCGAG CGCAGGGCCA 3240

GCAATGACCA GCGTCCCCAG GAGGTCCCAG CAGAGGCTCT GGCCCCGGCC CCAGTGGAAG 3300

TCCCAGCTCC AGCCCCGG 3318

INFORMATION FOR SEQ ID NO: 4

SEQUENCE CHARACTERISTICS:

LENGTH: 1171 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 4

GAGGAGGAGG AAGAGGAGGA TGAAGAGGCC GAGGAGGAGC GCCTGGCTCT GGAATGGGCC 60

CTGGGCGCGG ACGAGGACTT CCTGCTGGAG CACATCCGCA TCCTCAAGGT GCTGTGGTGC 120

TTCCTGATCC ATGTGCAGGG CAGTATCCGC CAGTTCGCCG CCTGCCTTGT GCTCACCGAC 180

TTCGGCATCG CAGTCTTCGA GATCCCGCAC CAGGAGTCTC GGGGCAGCAG CCAGCACATC 240

CTCTCCTCCC TGCGCTTTGT CTTTTGCTTC CCGCATGGCG ACCTCACCGA GTTTGGCTTC 300

CTCATGCCGG AGCTGTGTCT GGTGCTCAAG GTACGGCACA GTGAGAACAC GCTCTTCATT 360

ATCTCGGACG CCGCCAACCT GCACGAGTTC CACGCGGACC TGCGCTCATG CTTTGCACCC 420

CAGCACATGG CCATGCTGTG TAGCCCCATC CTCTACGGCA GCCACACCAG CCTGCAGGAG 480

TTCCTGCGCC AGCTGCTCAC CTTCTACAAG GTGGCTGGCG GCTGCCAGGA GCGCAGCCAG 540

GGCTGCTTCC CCGTCTACCT GGTCTACAGT GACAAGCGCA TGGTGCAGAC GGCCGCCGGG 600

GACTACTCAG GCAACATCGA GTGGGCCAGC TGCACACTCT GTTCAGCCGT GCGGCGCTCC 660

TGCTGCGCGC CCTCTGAGGC CGTCAAGTCC GCCGCCATCC CCTACTGGCT GTTGCTCACG 720

CCCCAGCACC TCAACGTCAT CAAGGCCGAC TTCAACCCCA TGCCCAACCG TGGCACCCAC 780

AACTGTCGCA ACCGCAACAG CTTCAAGCTC AGCCGTGTGC CGCTCTCCAC CGTGCTGCTG 840

GACCCCACAC GCAGCTGTAC CCAGCCTCGG GGCGCCTTTG CTGATGGCCA CGTGCTAGAG 900

CTGCTCGTGG GGTACCGCTT TGTCACTGCC ATCTTCGTGC TGCCCCACGA GAAGTTCCAC 960

TTCCTGCGCG TCTACAACCA GCTGCGGGCC TCGCTGCAGG ACCTGAAGAC TGTGGTCATC 1020

GCCAAGACCC CCGGGACGGG AGGCAGCCCC CAGGGCTCCT TTGCGGATGG CCAGCCTGCC 1080

GAGCGCAGGG CCAGCAATGA CCAGCGTCCC CAGGAGGTCC CAGCAGAGGC TCTGGCCCCG 1140

GCCCCAGTGG AAGTCCCAGC TCCAGCCCCG G 1171

INFORMATION FOR SEQ ID NO: 5

SEQUENCE CHARACTERISTICS:

LENGTH: 651 amino acids

TYPE: polypeptide

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 5

Met Thr Gly Gln Val Gly Ala Gln Thr Val Ser
1 5 10

Gly Gly Lys Arg Ser Ile Ala Gly Leu Thr Leu Val Arg Pro Leu Arg
15 20 25

Ser Val His Leu Leu Asp Met Ser Val Gln Val Ile Arg Pro Gly Glu
30 35 40

Ala Phe Pro Thr Ala Leu Ala Asp Val Arg Trp Asn Ser Pro Glu Lys
45 50 55

Lys Gly Gly Glu Asp Ser Trp Leu Ser Ala Ala Pro Cys Ile Arg Pro
60 65 70 75

Ser Ser Ser Pro Pro Thr Val Ala Pro Ala Ser Ala Ser Leu Pro Gln
80 85 90

Pro Ile Leu Ser Asn Gln Gly Ile Met Phe Val Gln Glu Glu Ala Leu
95 100 105

Ala Ser Ser Leu Ser Ser Thr Asp Ser Leu Thr Pro Glu His Gln Pro
110 115 120

Ile Ala Gln Gly Cys Ser Asp Ser Leu Glu Ser Ile Pro Ala Gly Gln

```
    125                 130                 135
Ala Ala Ser Asp Asp Leu Arg Asp Val Pro Gly Ala Val Gly Gly Ala
140                 145                 150                 155
Ser Pro Glu His Ala Glu Pro Glu Val Gln Val Val Pro Gly Ser Gly
                160                 165                 170
Gln Ile Ile Phe Leu Pro Phe Thr Cys Ile Gly Tyr Thr Ala Thr Asn
            175                 180                 185
Gln Asp Phe Ile Gln Arg Leu Ser Thr Leu Ile Trp Gln Ala Ile Glu
        190                 195                 200
Trp Gln Leu Pro Ala Trp Ile Glu Ala Ala Asn Gln Trp Glu Glu Gly
    205                 210                 215
Gln Gly Glu Gln Gly Glu Glu Glu Asp Glu Glu Glu Glu Glu Glu Glu
220                 225                 230                 235
Asp Val Ala Glu Asn Arg Tyr Phe Glu Met Gly Pro Pro Asp Val Glu
                240                 245                 250
Glu Glu Glu Gly Gly Gly Gln Gly Glu Glu Glu Glu Glu Glu Glu Glu
            255                 260                 265
Asp Glu Glu Ala Glu Glu Glu Arg Leu Ala Leu Glu Trp Ala Leu Gly
        270                 275                 280
Ala Asp Glu Asp Phe Leu Leu Glu His Ile Arg Ile Leu Lys Val Leu
    285                 290                 295
Trp Cys Phe Leu Ile His Val Gln Gly Ser Ile Arg Gln Phe Ala Ala
300                 305                 310                 315
Cys Leu Val Leu Thr Asp Phe Gly Ile Ala Val Phe Glu Ile Pro His
                320                 325                 330
Gln Glu Ser Trp Gly Ser Ser Gln His Ile Leu Ser Ser Leu Arg Phe
            335                 340                 345
Val Phe Cys Phe Pro His Gly Asp Leu Thr Glu Phe Gly Phe Leu Met
        350                 355                 360
Pro Glu Leu Cys Leu Val Leu Lys Val Arg His Ser Glu Asn Thr Leu
    365                 370                 375
Phe Ile Ile Ser Asp Ala Ala Asn Leu His Glu Phe His Ala Asp Leu
380                 385                 390                 395
Arg Ser Cys Phe Ala Pro Gln His Met Ala Met Leu Cys Ser Pro Ile
```

400 405 410

Leu Tyr Gly Ser His Thr Ser Leu Gln Glu Phe Leu Arg Gln Leu Leu

415 420 425

Thr Phe Tyr Lys Val Ala Gly Gly Cys Gln Glu Arg Ser Gln Gly Cys

430 435 440

Phe Pro Val Tyr Leu Val Tyr Ser Asp Lys Arg Met Val Gln Thr Ala

445 450 455

Ala Gly Asp Tyr Ser Gly Asn Ile Glu Trp Ala Ser Cys Thr Leu Cys

460 465 470 475

Ser Ala Val Arg Arg Ser Cys Cys Ala Pro Ser Glu Ala Val Lys Ser

480 485 490

Ala Ala Ile Pro Tyr Trp Leu Leu Leu Thr Pro Gln His Leu Asn Val

495 500 505

Ile Lys Ala Asp Phe Asn Pro Met Pro Asn Arg Gly Thr His Asn Cys

510 515 520

Arg Asn Arg Asn Ser Phe Lys Leu Ser Arg Val Pro Leu Ser Thr Val

525 530 535

Leu Leu Asp Pro Thr Arg Ser Cys Thr Gln Pro Arg Gly Ala Phe Ala

540 545 550 555

Asp Gly His Val Leu Glu Leu Leu Val Gly Tyr Arg Phe Val Thr Ala

560 565 570

Ile Phe Val Leu Pro His Glu Lys Phe His Phe Leu Arg Val Tyr Asn

575 580 585

Gln Leu Arg Ala Ser Leu Gln Asp Leu Lys Thr Val Val Ile Ala Lys

590 595 600

Thr Pro Gly Thr Gly Gly Ser Pro Gln Gly Ser Phe Ala Asp Gly Gln

605 610 615

Pro Ala Glu Arg Arg Ala Ser Asn Asp Gln Arg Pro Gln Glu Val Pro

620 625 630 635

Ala Glu Ala Leu Ala Pro Ala Pro Val Glu Val Pro Ala Pro Ala Pro

640 645 650

INFORMATION FOR SEQ ID NO: 6

SEQUENCE CHARACTERISTICS:

LENGTH: 390 amino acids

TYPE: polypeptide

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 6

```
Glu Glu Glu Glu Glu Glu
 1               5

Asp Glu Glu Ala Glu Glu Glu Arg Leu Ala Leu Glu Trp Ala Leu Gly
             10                  15                  20

Ala Asp Glu Asp Phe Leu Leu Glu His Ile Arg Ile Leu Lys Val Leu
         25                  30                  35

Trp Cys Phe Leu Ile His Val Gln Gly Ser Ile Arg Gln Phe Ala Ala
     40                  45                  50

Cys Leu Val Leu Thr Asp Phe Gly Ile Ala Val Phe Glu Ile Pro His
55                   60                  65                  70

Gln Glu Ser Trp Gly Ser Ser Gln His Ile Leu Ser Ser Leu Arg Phe
                 75                  80                  85

Val Phe Cys Phe Pro His Gly Asp Leu Thr Glu Phe Gly Phe Leu Met
             90                  95                  100

Pro Glu Leu Cys Leu Val Leu Lys Val Arg His Ser Glu Asn Thr Leu
         105                 110                 115

Phe Ile Ile Ser Asp Ala Ala Asn Leu His Glu Phe His Ala Asp Leu
     120                 125                 130

Arg Ser Cys Phe Ala Pro Gln His Met Ala Met Leu Cys Ser Pro Ile
135                  140                 145                 150

Leu Tyr Gly Ser His Thr Ser Leu Gln Glu Phe Leu Arg Gln Leu Leu
                 155                 160                 165

Thr Phe Tyr Lys Val Ala Gly Gly Cys Gln Glu Arg Ser Gln Gly Cys
             170                 175                 180

Phe Pro Val Tyr Leu Val Tyr Ser Asp Lys Arg Met Val Gln Thr Ala
         185                 190                 195

Ala Gly Asp Tyr Ser Gly Asn Ile Glu Trp Ala Ser Cys Thr Leu Cys
     200                 205                 210
```

```
Ser Ala Val Arg Arg Ser Cys Cys Ala Pro Ser Glu Ala Val Lys Ser
215                 220                 225                 230
Ala Ala Ile Pro Tyr Trp Leu Leu Leu Thr Pro Gln His Leu Asn Val
                235                 240                 245
Ile Lys Ala Asp Phe Asn Pro Met Pro Asn Arg Gly Thr His Asn Cys
            250                 255                 260
Arg Asn Arg Asn Ser Phe Lys Leu Ser Arg Val Pro Leu Ser Thr Val
        265                 270                 275
Leu Leu Asp Pro Thr Arg Ser Cys Thr Gln Pro Arg Gly Ala Phe Ala
    280                 285                 290
Asp Gly His Val Leu Glu Leu Leu Val Gly Tyr Arg Phe Val Thr Ala
295                 300                 305                 310
Ile Phe Val Leu Pro His Glu Lys Phe His Phe Leu Arg Val Tyr Asn
                315                 320                 325
Gln Leu Arg Ala Ser Leu Gln Asp Leu Lys Thr Val Val Ile Ala Lys
            330                 335                 340
Thr Pro Gly Thr Gly Gly Ser Pro Gln Gly Ser Phe Ala Asp Gly Gln
        345                 350                 355
Pro Ala Glu Arg Arg Ala Ser Asn Asp Gln Arg Pro Gln Glu Val Pro
    360                 365                 370
Ala Glu Ala Leu Ala Pro Ala Pro Val Glu Val Pro Ala Pro Ala Pro
375                 380                 385                 390
```

INFORMATION FOR SEQ ID NO: 7

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 7

```
CTTGAGGATG CGGATGTGCT   20
```

INFORMATION FOR SEQ ID NO: 8

SEQUENCE CHARACTERISTICS:

LENGTH: 18 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 8

CCATGGGGTG AGTGTCCT 18

INFORMATION FOR SEQ ID NO: 9

SEQUENCE CHARACTERISTICS:

LENGTH: 18 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 9

AGGACACTCA CCCCATGG 18

INFORMATION FOR SEQ ID NO: 10

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 10

GTATGGGACA GGGGCAGAAA 20

INFORMATION FOR SEQ ID NO: 11

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 11

TTTCTAAAGA CCATTGGGAG 20

INFORMATION FOR SEQ ID NO: 12

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 12

CCATTTTAAA GTAGCGGTTC 20

INFORMATION FOR SEQ ID NO: 13

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 13

AGGAGAGAAA GGTGAGCCAA 20

INFORMATION FOR SEQ ID NO: 14

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 14

GTAGATCCTG AGGTTGACCA 20

INFORMATION FOR SEQ ID NO: 15

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 15

TGTGAGCATT TCTGGCCTTC 20

INFORMATION FOR SEQ ID NO: 16

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 16

TGAAGACGCC AGAGAAGCAG 20

INFORMATION FOR SEQ ID NO: 17

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 17

GCCTCACAAG TGTCAGACCT 20

INFORMATION FOR SEQ ID NO: 18

SEQUENCE CHARACTERISTICS:

LENGTH: 18 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 18

AGAAGGGTGG TGAAGACT 18

INFORMATION FOR SEQ ID NO: 19

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 19

CTTGGTTAGA GAGGATGGGC 20

INFORMATION FOR SEQ ID NO: 20

SEQUENCE CHARACTERISTICS:

LENGTH: 20 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 20

GCCCATCCTC TCTAACCAAG 20

INFORMATION FOR SEQ ID NO: 21

SEQUENCE CHARACTERISTICS:

LENGTH: 15202 nucleic acids

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

FEATURE:

NAME/KEY:

LOCATION:

IDENTIFICATION METHOD:

OTHER INFORMATION: /note="N is unknown or other"

SEQUENCE DESCRIPTION: SEQ ID NO: 21

GATCCGAGCT CAATTAACCC TCACTAAAGG GAGTCGACTC GATCCTTAAA ATATTCATAT 60

CTCCTGGACA ACCTGTGGCC ATAGTGCCTG ACTGTAAACC CAAAGGGTTT GCCTTTGCCA 120

GTGTAGCCCA GCCTGGTGTC TGCTGCCCCT CGCGGTGTCT GTGCACCTGC CACGATGCTG 180

ACCAGACACC CTTAACCAGG TTCACCCATC GCCTGGGCCT GGAGCAGTCC CCCTGATGCT 240

CTGATTGGTC CTTGGACCTT CTGTTCTCCC AAAATCCCAG GTCAGAAAAT ACCTGGAAGT 300

CTATTTGTGT CCCACCTCCC TCTTTGTGGC CGCAAGTGCC CCTTCCTCCA CACAGTCACA 360

AGACCATGAG ATGCCATCTC CTCCCCTCCT GGGCTGCAGA CTTTGGGAAG CTCCCAGGCC 420

ACAGAGGTGT CAGCTCCTGT CCAGGCCCTT GGGACCTTCC CTCATTCAAC CACCCTACCC 480

AACCCCCCAC TGCCTGCCAG CCACCACTCC CTCCCACATT TGCAGGCGGG GGCCCTGCCC 540

TCTCCTGCCG CTGGTTCCCC TACCCAGGAG GCTCTCCCAT CGCTCTTTTG AGAGTCTGCC 600

TCCCACCTCT AACTGGGGGC TTAGTTCAAG TTGCCCCCTT ACCCTAGTCC CAGCTGCCCA 660

AGAGCTTGCT GCCTCCTGTT CTTGGTGAGG GACTCCAGAG ACAGATGTGA GACCTCCCTG 720

GACCCCTCCA AGGCATTCCC AGGTCACTTC CATGAGTAGT GAAGAACCGC CTCTGAGCAG 780

GCTGAGCCTC CCTCAGCCTA TGGTGTCCTC ACGTGGCTTG GCCCACAGCA GGTGCTCACG 840

CCTCCTCCTC AGCAGAGCCT ACCATCCTCC TGCCATGCTC ACCAGTCCCC ATGCTGATAG 900

CCATCACCAG TCCCCATGCT GATAGCCATC ACCAGTCCCC ATGCTGATAG CCACTTTCTG 960

GATGCTCTAG GTCTGTCTGG ATGACACAGT GACCACAGAG AAGGAGCTGG ACACTGTGGA 1020

AGTGCTGAAA GCAATTCAGA AAGCCAAGGA GGTCAAGTCC AAACTGAGCA ACCCAGAGAA 1080

GAAGGTGGGT TTGTGTGGCA GGTGGGAGGG CAGTGGTGCA GAGCCAGCCG GGATAGGAGC 1140

CAGTTCGGGG GGCTTGGGCC ATGGGACTGC TCAGGGCTGC CGAGTCCCAG CTGCGCCCCT 1200

CCCTGGCTGC ATGACCTCGG GCAAGTCGCG GCCTCTCTGT TCTCTGTGGG GTGGGGACAG 1260

TGGTAGTTCC TGCTCTAAGG ATATGATGAG ACCATCTTTA CCACCCAGTT GGTGGGAACC 1320

GTTGCGCTCC CTCCTCACAC CCCTGGCCTT GGGGAGCTCT GTGCTTCCTC TTCTCTCCCG 1380

GGCTGACTCA AGCACTCGTC CTCAGGGTGG TGAAGACTCC CGGCTCTCAG CTGCCCCCTG 1440

CATCAGACCC AGCAGCTCCC CTCCCACTGT GGCTCCCGCA TCTGCCTCCC TGCCCCAGCC 1500

CATCCTCTCT AACCAAGGTA ATCGTGTATG TATCTTGCTT CTAGTGGAGC CACACAGCCC 1560

TGCCTGGGCC CCCTGGCTGG GCTGGGGTTG GGGGAGAGGT GCCAGCACCT GCTTCCAACA 1620

GGGTCAGACA CAGGGAGGGC AGTGCCTTCT GCAGGCTGGT CCTCGCGGGG GGACACATGG 1680

CAGGGGTGCC TGGCCTGATG CCAGCTGTTG CTTGCTTGGT GAGGACTCCC AATTGCTCTG 1740

ATGCCCACAT CCAGCTCCTC TAGGAGACCG CAGGGTGTCT GACAGGCCCT GAGGCTGCCC 1800

TCTGAACAGG CTCGGGGCTG TTGGCTCATG GGACCCATTC CCTCACCGGC AGCACAAGCA 1860

GGTTGGCTCC TGGTTACAGG AAGCCGGGCT TGTGACTTTA CTGTCTGGAG CCCGAATCCC 1920

TGTGCAGGGA AAAGCTTGCT TTTATCACTG CCTCATCTCT GTGGGGTGAC CCAGCCCCAG 1980

AACACCATGT TTGTGGGGCC AAGATGGGCC ATCTCTGTCC CTGTGGACCC ATGGAAGACC 2040

AGGCCCATTC GTCTGCCCAC TATCTTAGCG TTTTCAAAGG GCTTTCACCT CTGAACCCAG 2100

GCATCCTCGG AGATGAGTGA GTGAAGCAGG TCTCATGAGC GTGTCTGCTG GCCCGGCCCC 2160

CACGGAAGAG GGGAGGGTGT GCCGTCCCGA GTGGAGCCGA GGCTCGGGAC ACGCAGGAAA 2220

GGACGCCGCC TGCCCGGGCT CCTGGAGACG CAGAACTTGG TGTGAGGTCT TGGGAAAACA 2280

GTTCAACCCG ATGTTTTAAG AGCCAGAAAA ACATTCCCAC CCCTTGACCT GGTAACCCCA 2340

CTGGTGGGGA TTTTCTCTTA GAGGGATAAG ATACCGGGAA GGGGAGGTGA AATGCTCACC 2400

ACTGCCAAAA CACGGGCTGC AACTGCAACA TCGGAGGATG AGAGGGAGAG TCGGCTGTGG 2460

TGCAGAATGC TCAGCAGCCC TCCCAGCAGG GACAGGAAGA CTGGGCAGGA AGAGGGGAGA 2520

AGCATTCAAG TTAAGGCAAA AGGCCCAACG CAGAGCAGCA CACTGAGGTC ACACCTGTGA 2580

GATGTGGAAG AGAATTCCTG AGCGTGGAGC GATGGGGTTA GGTGCCAGGA TGATTGCCCA 2640

TTTTGCTTCT GTCAGACTCT TGACTAAGGA TTTCTGGTTG CATTTTATTA CATAAAAGCC 2700

AGGGAGGTTA TATCACGGTG AGAAAGCTTC CCTGACGCCG CCTCCTGTAG CGCAGCCAAG 2760

CGAGCCTGTG GAGGTACCAT ATGACTGTAG GCCTCTGGGG ACAGGGAGCT GCATCTGCTT 2820

CTCAAGGCCA GGGACACAGC CATTTCTGCC AGCATCTGTT GATCAGTGAG TGAGTGAGTG 2880

GGCAGGTAGA GCAGGAGCCA GTGAAGAGCA GGCCCTGGAT GGGTGGGGAT GCACCATGTC 2940

CCCAGGCTGC AGCTGCAGGC AGCCCCCCAC ATTGTCGGAG AAGCCTCTGC ACCAGCTCAG 3000

CCCCCTCCTC ACTCCCCTTG TGCCCTGGGG ACACTCTGCA GAGGGGCACT CTGCAGTCTG 3060

TCCCCGCCAT CGCTGGACTT CTGGACATGG CCTCCAGATT TGCACCTCTT AAATAAATCT 3120

GCAGTGGATG TCTTTGTGTG CACCTCTCTT TCCTTTTGGT GAGAAACAGC AAAGATCGGA 3180

CCCCTAAGGA CTCTCCTGAT GTCTCCGCTC TATCCGCTGA GTGCCCTTTC TGACCACTTG 3240

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AAGGGTGGAN TTCCTGAANG GG 15202

INFORMATION FOR SEQ ID NO: 22

SEQUENCE CHARACTERISTICS:

LENGTH: 1070 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO: 22

Val Cys Leu Asp Asp Thr Val Thr Thr Glu Lys Glu Leu Asp Thr Val
1 5 10 15

Glu Val Leu Lys Ala Ile Gln Lys Ala Lys Glu Val Lys Ser Lys Leu
20 25 30

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Ser Asn Pro Glu Lys Lys Gly Gly Glu Asp Ser Arg Leu Ser Ala Ala
          35                  40                  45
Pro Cys Ile Arg Pro Ser Ser Ser Pro Pro Thr Val Ala Pro Ala Ser
      50                  55                  60
Ala Ser Leu Pro Gln Pro Ile Leu Ser Asn Gln Gly Ile Met Phe Val
65                    70                  75                  80
Gln Glu Glu Ala Leu Ala Ser Ser Leu Ser Ser Thr Asp Ser Leu Thr
                  85                  90                  95
Pro Glu His Gln Pro Ile Ala Gln Gly Cys Ser Asp Ser Leu Glu Ser
             100                 105                 110
Ile Pro Ala Gly Gln Ala Ala Ser Asp Asp Leu Arg Asp Val Pro Gly
         115                 120                 125
Ala Val Gly Gly Ala Ser Pro Glu His Ala Glu Pro Glu Val Gln Val
     130                 135                 140
Val Pro Gly Ser Gly Gln Ile Ile Phe Leu Pro Phe Thr Cys Ile Gly
145                   150                 155                 160
Tyr Thr Ala Thr Asn Gln Asp Phe Ile Gln Arg Leu Ser Thr Leu Ile
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Arg Gln Ala Ile Glu Arg Gln Leu Pro Ala Trp Ile Glu Ala Ala Asn
             180                 185                 190
Gln Arg Glu Glu Gly Gln Gly Glu Gln Gly Glu Glu Glu Asp Glu Glu
         195                 200                 205
Glu Glu Glu Glu Glu Asp Val Ala Glu Asn Arg Tyr Phe Glu Met Gly
     210                 215                 220
Pro Pro Asp Val Glu Glu Glu Glu Gly Gly Gly Gln Gly Glu Glu Glu
225                   230                 235                 240
Glu Glu Glu Glu Glu Asp Glu Glu Ala Glu Glu Glu Arg Leu Ala Leu
                  245                 250                 255
Glu Trp Ala Leu Gly Ala Asp Glu Asp Phe Leu Leu Glu His Ile Arg
             260                 265                 270
Ile Leu Lys Val Leu Trp Cys Phe Leu Ile His Val Gln Gly Ser Ile
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Arg Gln Phe Ala Ala Cys Leu Val Leu Thr Asp Phe Gly Ile Ala Val
     290                 295                 300
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Phe Gly Phe Leu Met Pro Glu Leu Cys Leu Val Leu Lys Val Arg His
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Phe His Ala Asp Leu Arg Ser Cys Phe Ala Pro Gln His Met Ala Met
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Arg Ser Gln Gly Cys Phe Pro Val Tyr Leu Val Tyr Ser Asp Lys Arg
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Met Val Gln Thr Ala Ala Gly Asp Tyr Ser Gly Asn Ile Glu Trp Ala
435 440 445
Ser Cys Thr Leu Cys Ser Ala Val Arg Arg Ser Cys Cys Ala Pro Ser
450 455 460
Glu Ala Val Lys Ser Ala Ala Ile Pro Tyr Trp Leu Leu Leu Thr Pro
465 470 475 480
Gln His Leu Asn Val Ile Lys Ala Asp Phe Asn Pro Met Pro Asn Arg
485 490 495
Gly Thr His Asn Cys Arg Asn Arg Asn Ser Phe Lys Leu Ser Arg Val
500 505 510
Pro Leu Ser Thr Val Leu Leu Asp Pro Thr Arg Ser Cys Thr Gln Pro
515 520 525
Arg Gly Ala Phe Ala Asp Gly His Val Leu Glu Leu Leu Val Gly Tyr
530 535 540
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545 550 555 560
Leu Arg Val Tyr Asn Gln Leu Arg Ala Ser Leu Gln Asp Leu Lys Thr
565 570 575

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    610                 615                 620
Pro Ala Pro Ala Pro Ala Ala Ala Ser Ala Ser Gly Pro Ala Lys Thr
625                 630                 635                 640
Pro Ala Pro Ala Glu Ala Ser Thr Ser Ala Leu Val Pro Glu Glu Thr
                645                 650                 655
Pro Val Glu Ala Pro Ala Pro Pro Pro Ala Glu Ala Pro Ala Gln Tyr
            660                 665                 670
Pro Ser Glu His Leu Ile Gln Ala Thr Ser Glu Glu Asn Gln Ile Pro
        675                 680                 685
Ser His Leu Pro Ala Cys Pro Ser Leu Arg His Val Ala Ser Leu Arg
    690                 695                 700
Gly Ser Ala Ile Ile Glu Leu Phe His Ser Ser Ile Ala Glu Val Glu
705                 710                 715                 720
Asn Glu Glu Leu Arg His Leu Met Trp Ser Ser Val Val Phe Tyr Gln
                725                 730                 735
Thr Pro Gly Leu Glu Val Thr Ala Cys Val Leu Leu Ser Thr Lys Ala
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Val Tyr Phe Val Leu His Asp Gly Leu Arg Arg Tyr Phe Ser Glu Pro
        755                 760                 765
Leu Gln Asp Phe Trp His Gln Lys Asn Thr Asp Tyr Asn Asn Ser Pro
    770                 775                 780
Phe His Ile Ser Gln Cys Phe Val Leu Lys Leu Ser Asp Leu Gln Ser
785                 790                 795                 800
Val Asn Val Gly Leu Phe Asp Gln His Phe Arg Leu Thr Gly Ser Thr
                805                 810                 815
Pro Met Gln Val Val Thr Cys Leu Thr Arg Asp Ser Tyr Leu Thr His
            820                 825                 830
Cys Phe Leu Gln His Leu Met Val Val Leu Ser Ser Leu Glu Arg Thr
        835                 840                 845
```

```
Pro Ser Pro Glu Pro Val Asp Lys Asp Phe Tyr Ser Glu Phe Gly Asn
    850                 855                 860
Lys Thr Thr Gly Lys Met Glu Asn Tyr Glu Leu Ile His Ser Ser Arg
865                 870                 875                 880
Val Lys Phe Thr Tyr Pro Ser Glu Glu Glu Ile Gly Asp Leu Thr Phe
                885                 890                 895
Thr Val Ala Gln Lys Met Ala Glu Pro Glu Lys Ala Pro Ala Leu Ser
            900                 905                 910
Ile Leu Leu Tyr Val Gln Ala Phe Gln Val Gly Met Pro Pro Pro Gly
        915                 920                 925
Cys Cys Arg Gly Pro Leu Arg Pro Lys Thr Leu Leu Leu Thr Ser Ser
    930                 935                 940
Glu Ile Phe Leu Leu Asp Glu Asp Cys Val His Tyr Pro Leu Pro Glu
945                 950                 955                 960
Phe Ala Lys Glu Pro Pro Gln Arg Asp Arg Tyr Arg Leu Asp Asp Gly
                965                 970                 975
Arg Arg Val Arg Asp Leu Asp Arg Val Leu Met Gly Tyr Gln Thr Tyr
            980                 985                 990
Pro Gln Ala Leu Thr Leu Val Phe Asp Asp Val Gln Gly His Asp Leu
        995                1000                1005
Met Gly Ser Val Thr Leu Asp His Phe Gly Glu Val Pro Gly Gly Pro
   1010                1015                1020
Ala Arg Ala Ser Gln Gly Arg Glu Val Gln Trp Gln Val Phe Val Pro
1025                1030                1035                1040
Ser Ala Glu Ser Arg Glu Lys Leu Ile Ser Leu Leu Ala Arg Gln Trp
                1045                1050                1055
Glu Ala Leu Cys Gly Arg Glu Leu Pro Val Glu Leu Thr Gly
            1060                1065                1070
```

WHAT IS CLAIMED IS:

CLAIMS

1. A DNA molecule encoding for a polypeptide including an amino acid sequence which is receptive to imidazoline compounds, said DNA molecule containing a DNA sequence with at least 75% sequence similarity with the DNA sequence shown in SEQ ID No. 4.

2. A DNA molecule according to claim 1, containing a DNA sequence with at least 75% sequence similarity with the DNA sequence shown in SEQ ID No. 2.

3. A DNA molecule according to claim 2, containing a DNA sequence with at least 75% sequence similarity with the DNA sequence of SEQ ID No. 3.

4. A DNA molecule according to claim 3, containing a DNA sequence with at least 75% sequence similarity with the DNA sequence of SEQ ID No. 1.

5. A DNA molecule according to any one of claims 1 to 4, containing a DNA sequence with at least 80% sequence similarity with the sequence of said SEQ ID No.

6. A DNA molecule according to any one of claims 1 to 4, containing a DNA sequence with at least 85% sequence similarity with the sequence of said SEQ ID No.

7. A DNA molecule according to any one of claims 1 to 4, containing a DNA sequence with at least 90% sequence similarity with the sequence of said SEQ ID No.

8. A DNA molecule according to any one of claims 1 to 4, containing a DNA sequence with at least 95% sequence similarity with the sequence of said SEQ ID No.

9. A DNA molecule according to claim 1, which is deposited with the ATCC under deposit accession no. ATCC 209217.

10. A genomic DNA molecule encoding for a polypeptide including an amino acid sequence which is receptive to imidazoline compounds, and wherein exon portions of said genomic DNA molecule include the DNA sequence as defined in claim 1.

11. A genomic DNA molecule according to claim 10, which is deposited with the ATCC under deposit accession no. ATCC 209216.

12. A 1110 bp ApaI-EcoRI restriction fragment of the DNA molecule according to claim 1.

13. A 1.85 kb EcoRI restriction fragment of the DNA molecule according to claim 4.

14. A vector containing a DNA sequence as defined in any one of claims 1-13.

15. A host cell transfected with a vector as defined in claim 14.

16. An isolated polypeptide including a site which is receptive to imidazoline compounds, said polypeptide containing an amino acid sequence with at least 80% sequence similarity with the amino acid sequence shown in SEQ ID No. 6.

17. A polypeptide as defined in claim 16, having a molecular weight of about 35 to 45 kDa.

18. A polypeptide as defined in claim 17, having a molecular weight of about 37 kDa.

19. An isolated polypeptide including a site which is receptive to imidazoline compounds, said polypeptide containing an amino acid sequence with at least 80% sequence similarity with the amino acid sequence shown in SEQ ID No. 5.

20. A polypeptide as defined in claim 19, having a molecular weight of about 60 to 85 kDa.

21. A polypeptide as defined in claim 20, having a molecular weight of about 70 kDa.

22. A fragment of the amino acid sequence shown in SEQ ID No. 5 or 6, which fragment is receptive to imidazoline compounds.

23. A polypeptide according to any one of claims 16 to 22, which is immunoreactive with at least one of Reis antiserum and Dontenwill antiserum.

24. A polypeptide according to any one of claims 16 to 23, which is a human polypeptide.

25. A method of producing an isolated polypeptide including an amino acid sequence which is receptive to imidazoline compounds, said method comprising:

transfecting a host cell with a vector as defined in claim 14; and

culturing the transfected host cell in a culture medium to express the polypeptide.

26. An isolated polypeptide including an amino acid sequence which is receptive to imidazoline compounds, which polypeptide is expressed by the method of claim 25.

27. A method of screening for a ligand of an imidazoline receptor, which method comprises:

culturing a host cell as defined in claim 15 in a culture medium to express a polypeptide including an amino acid sequence which is receptive to imidazoline compounds;

contacting said polypeptide with a labelled ligand for the imidazoline receptor under conditions effective to bind the labelled ligand thereto;

contacting said polypeptide with a candidate ligand; and

detecting any displacement of the labelled ligand from said polypeptide, wherein displacement signifies that the candidate ligand is a ligand for the imidazoline receptor.

28. The method of claim 27, wherein said contacting steps are performed in an intact cultured host cell.

29. The method of claim 27, further comprising isolating the cell membrane of said cultured host cell prior to performing said contacting steps.

30. The method of claim 27, wherein said contacting of said imidazoline receptive polypeptide with said candidate ligand is conducted at a plurality of candidate ligand concentrations.

31. The method of claim 27, wherein the labelled ligand is radiolabelled.

32. A method of obtaining a DNA material encoding a polypeptide which is receptive to imidazoline compounds, said method comprising:

providing a labelled DNA probe by labelling a DNA molecule identical or complementary to a DNA molecule as defined in any one of claims 1 to 9 or a restriction fragment thereof;

contacting said DNA probe with genetic material suspected of encoding said imidazoline receptive polypeptide;

hybridizing said DNA probe and said genetic material under stringent hybridization conditions;

identifying any portion of the genetic material which hybridizes to said DNA probe; and

isolating said identified material.

33. A method according to claim 32, wherein the genetic material is derived from a library selected from the group consisting of RNA library, cDNA library and genomic DNA library.

34. A method according to claim 33, wherein said library is a human library.

35. A method according to claim 32, wherein the labelled DNA probe is provided by labelling a restriction fragment according to claim 12 or 13.

36. A method of raising antibodies immunoreactive with a polypeptide which is receptive to an imidazoline compound, which method comprises:

injecting an animal with a polypeptide as defined in any one of claims 16 to 24 and 26; and

isolating antibodies produced by the animal.

FIG. 1A

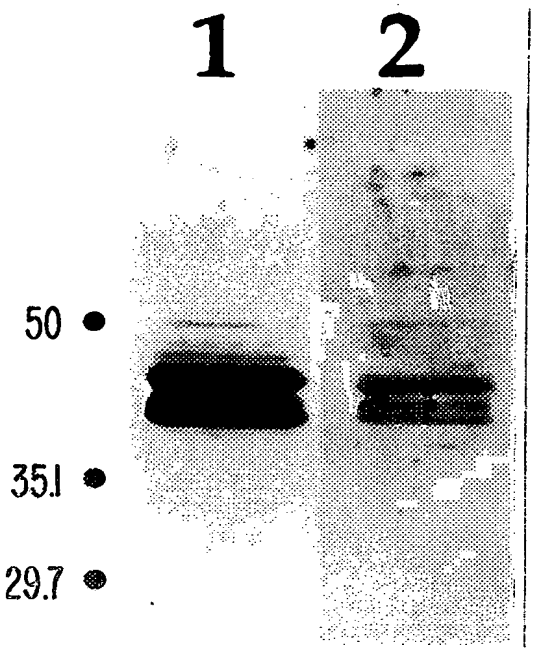


FIG. 1B

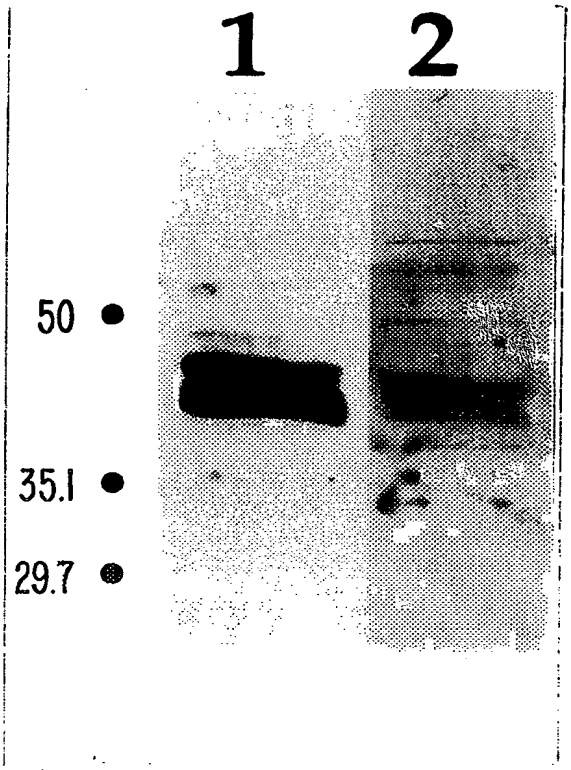
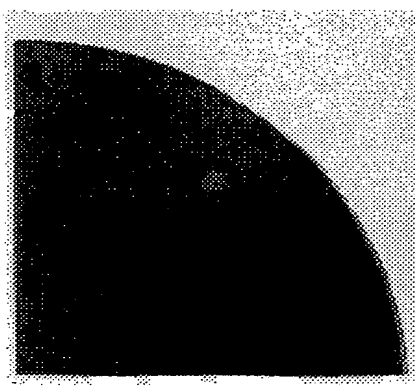


FIG. 2A



REIS AB
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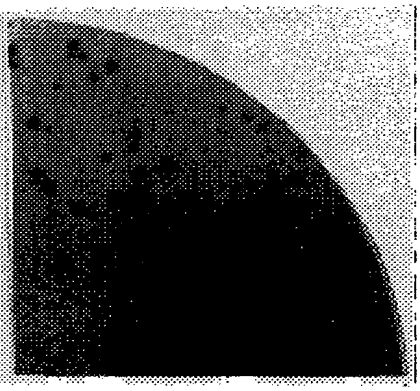
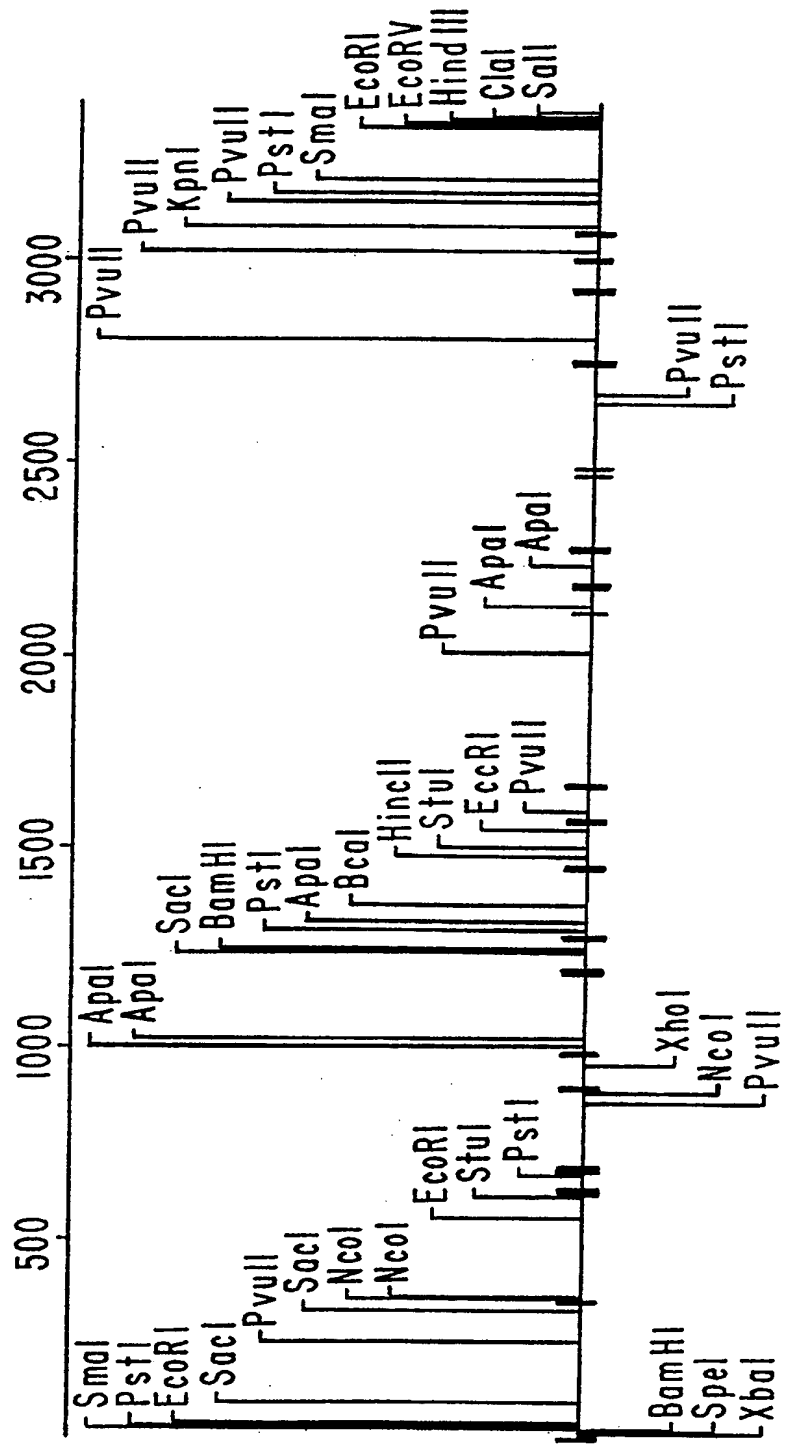
FIG. 2B



DONTEWILL AB
1:20,000 DILUTION

FIG. 3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1000
1500
2000
2500
3000
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PstI
EcoRI
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SacI
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StuI
PstI
ApaI
ApaI
SacI
BamHI
PstI
ApaI
BcoI
HincII
StuI
EccRI
PvuII
PvuII
ApaI
ApaI
XhoI
NcoI
PvuII
PvuII
PstI
PvuII
KpnI
PvuII
SmaI
PstI
EcoRI
EcoRV
HindIII
ClaI
SalI
BamHI
SpeI
XbaI

FIG. 4

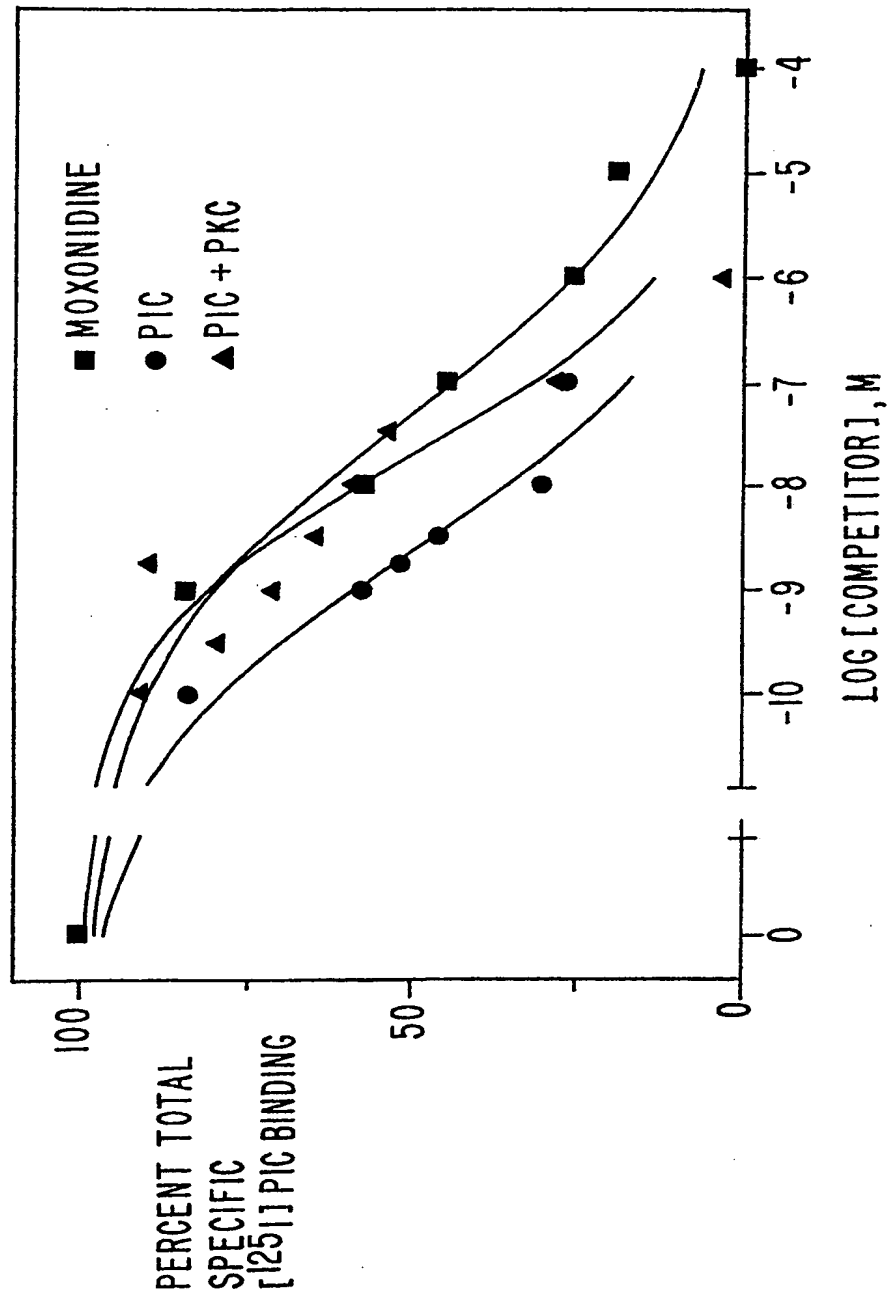
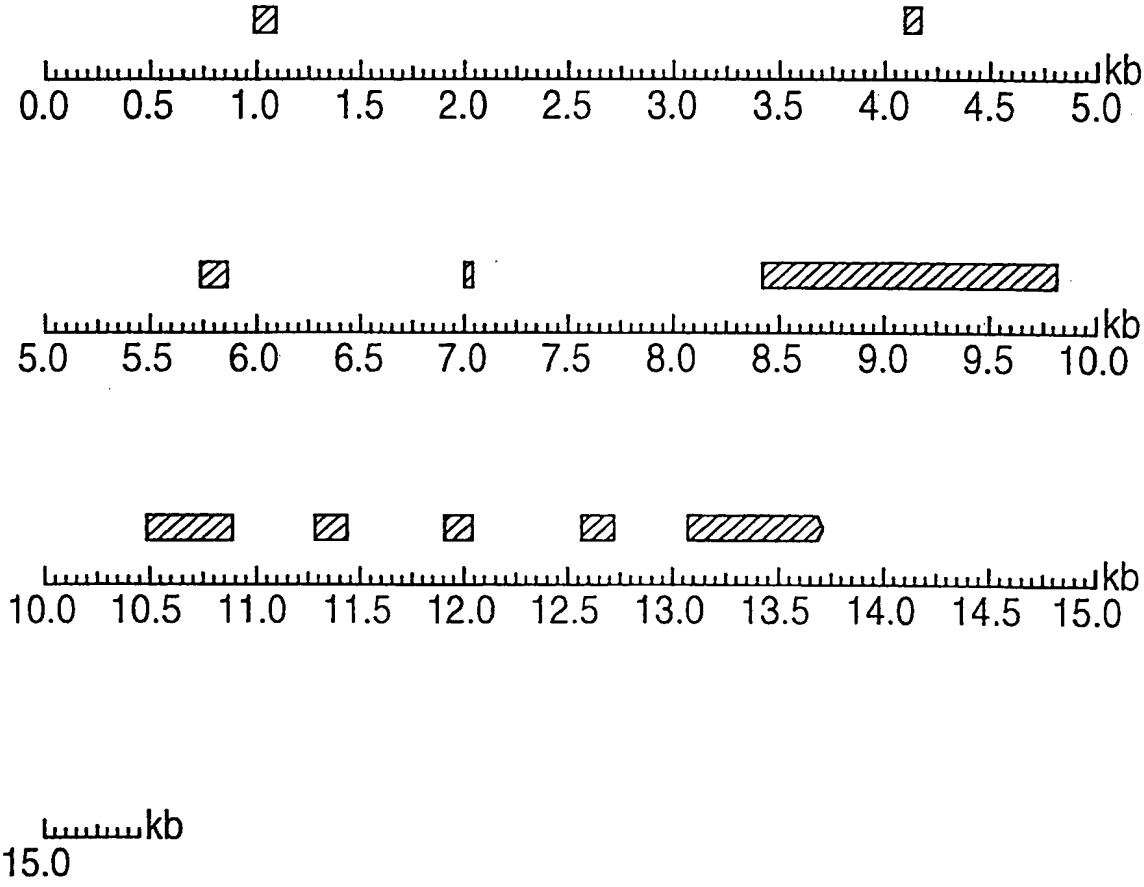

MOXONIDINE
PIC
PIC + PKC
PERCENT TOTAL SPECIFIC [125I] PIC BINDING
100
50
0
0
-10
-9
-8
-7
-6
-5
-4
LOG [COMPETITOR], M

FIG. 5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kb
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kb
10.0 10.5 11.0 11.5 12.0 12.5 13.0 13.5 14.0 14.5 15.0
kb
15.0
KEY:
INITIAL EXON
INTERNAL EXON
TERMINAL EXON
SINGLE-EXON GENE
OPTIMAL EXON
SUBOPTIMAL EXON

FIG. 6A

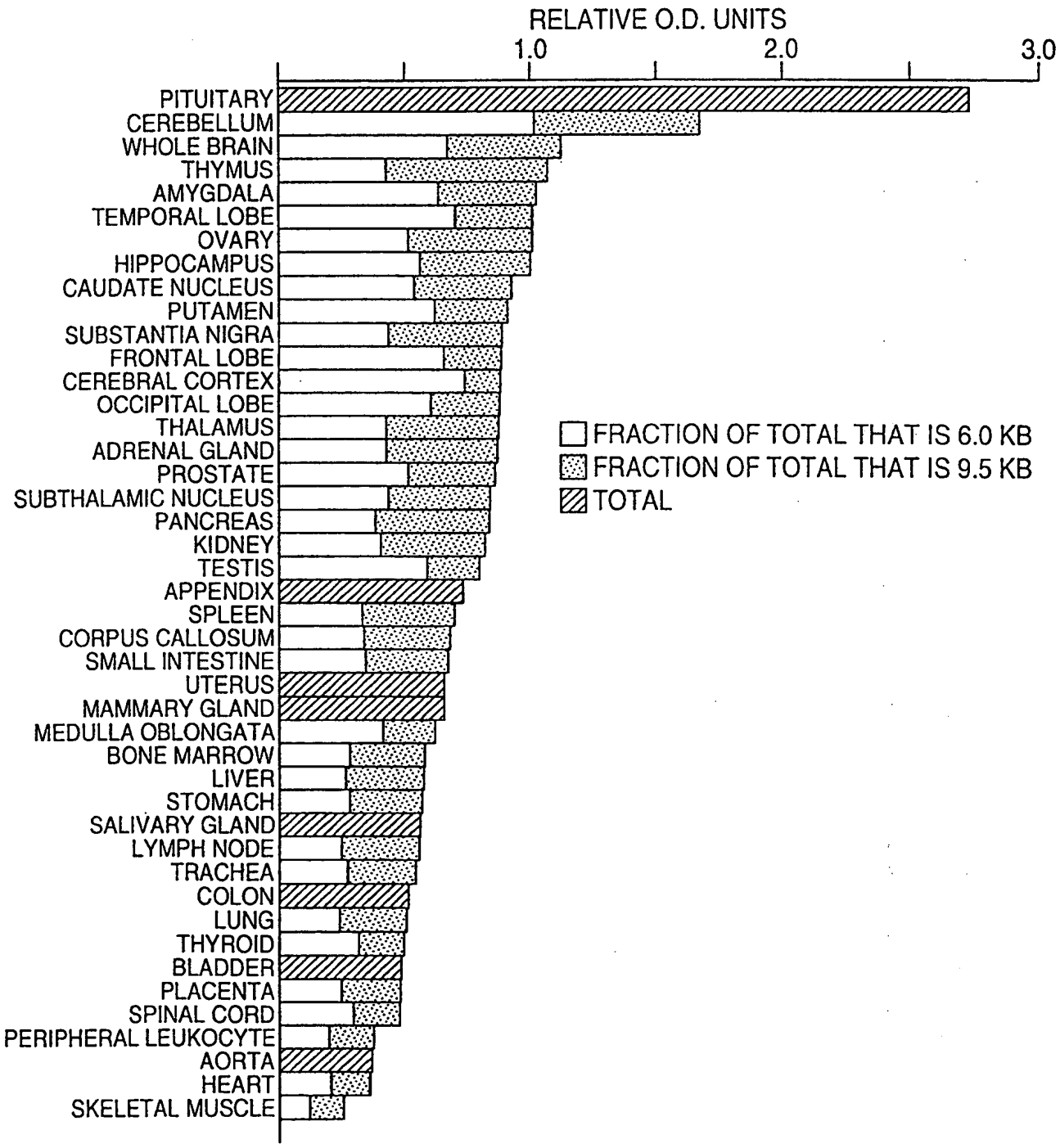
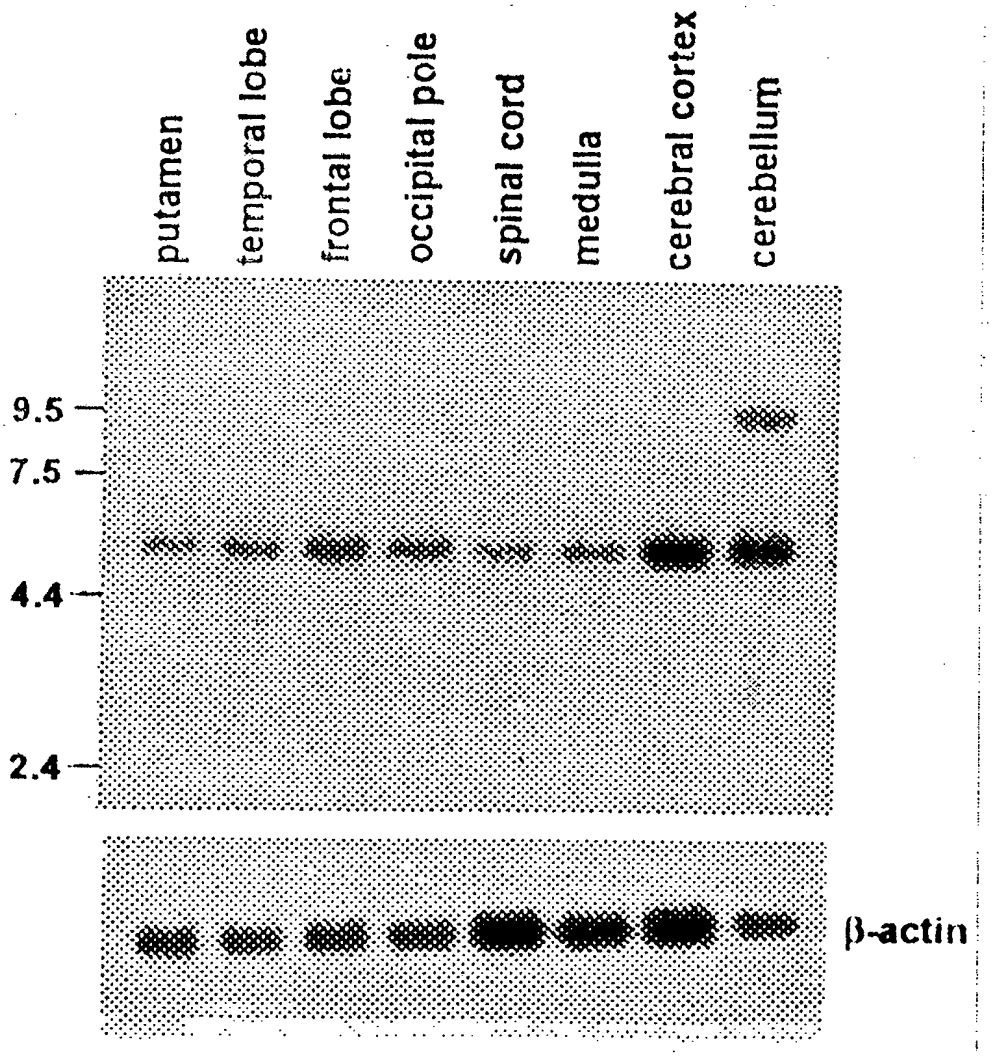

RELATIVE O.D. UNITS
1.0
2.0
3.0
PITUITARY
CEREBELLUM
WHOLE BRAIN
THYMUS
AMYGDALA
TEMPORAL LOBE
OVARY
HIPPOCAMPUS
CAUDATE NUCLEUS
PUTAMEN
SUBSTANTIA NIGRA
FRONTAL LOBE
CEREBRAL CORTEX
OCCIPITAL LOBE
THALAMUS
ADRENAL GLAND
PROSTATE
SUBTHALAMIC NUCLEUS
PANCREAS
KIDNEY
TESTIS
APPENDIX
SPLEEN
CORPUS CALLOSUM
SMALL INTESTINE
UTERUS
MAMMARY GLAND
MEDULLA OBLONGATA
BONE MARROW
LIVER
STOMACH
SALIVARY GLAND
LYMPH NODE
TRACHEA
COLON
LUNG
THYROID
BLADDER
PLACENTA
SPINAL CORD
PERIPHERAL LEUKOCYTE
AORTA
HEART
SKELETAL MUSCLE
FRACTION OF TOTAL THAT IS 6.0 KB
FRACTION OF TOTAL THAT IS 9.5 KB
TOTAL

FIG. 6B


putamen
temporal lobe
frontal lobe
occipital pole
spinal cord
medulla
cerebral cortex
cerebellum
9.5
7.5
4.4
2.4
β-actin

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/15695

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 A61K38/17 C12N5/10 C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL Emest5:Hs1228705 Accession number AA428250, 25 May 1997 "WashU-Merck EST Project 1997" XP002064993	19,24
Y	Unpublished see abstract	20-22

X	DATABASE EMBL Emest5:Hs1228705 Accession number AA428250, 25 May 1997 "WashU-Merck EST Project 1997" XP002064994 Unpublished see abstract	2,5-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 1998	15/06/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Halle, F

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INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 97/15695

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL Emest4:Hs1190779 Accession number AA287493, 12 April 1997 "National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index" XP002064995 Unpublished see abstract ---	4-8
X	DATABASE EMBL Emest4:Hs1190779 Accession number AA287493, 12 April 1997 "National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index" XP002064996 Unpublished see abstract ---	3,5-8
X	DATABASE EMBL Emest13:Mmw3021 Accession number W91302, 9 July 1996 "The WashU-HHMI Mouse EST Project" XP002064997 see abstract ---	3,5-7
X	DATABASE EMBL R54u006:Rs782 Accession number H31782, 30 September 1995 "Comparative expressed-sequence-tag analysis of differential gene expression profiles in PC-12 cells before and after nerve growth factor treatment" XP002064998 Unpublished see abstract & Proc. Natl. Acad. Sci. USA 92:8303-8307 (1995) ---	2,5
X	DATABASE EMBL Emest10:Hsc1sa031 Accession number F06998, 15 February 1995 "The Genexpress cDNA program" XP002064999	19,24
Y	Unpublished see abstract --- -/--	20-22

1

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INTERNATIONAL SEARCH REPORT

Int. .tional Application No
PCT/US 97/15695

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL Emest:Hs1442 Accession number T06144, 2 September 1993 "3,400 new expressed sequence tags identify diversity of transcripts in human brain" XP002065000	16,24
Y	see abstract & Nat. Genet. 4:256-267 (1993)	10,12, 14,15, 17,18,22
X	DATABASE EMBL Emest5:Hs1442 Accession number T06144, 2 September 1993 "3,400 new expressed sequence tags identify diversity of transcripts in human brain" XP002065001	1,5-7
Y	see abstract & Nat. Genet. 4:256-267 (1993)	10,12, 14,15, 17,18
E	WO 97 31945 A (UNIV MISSISSIPPI MEDICAL CENTE, 04.09.1997) 4 September 1997 see the whole document	1-36

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INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 97/15695

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9731945 A	04-09-97	NONE	